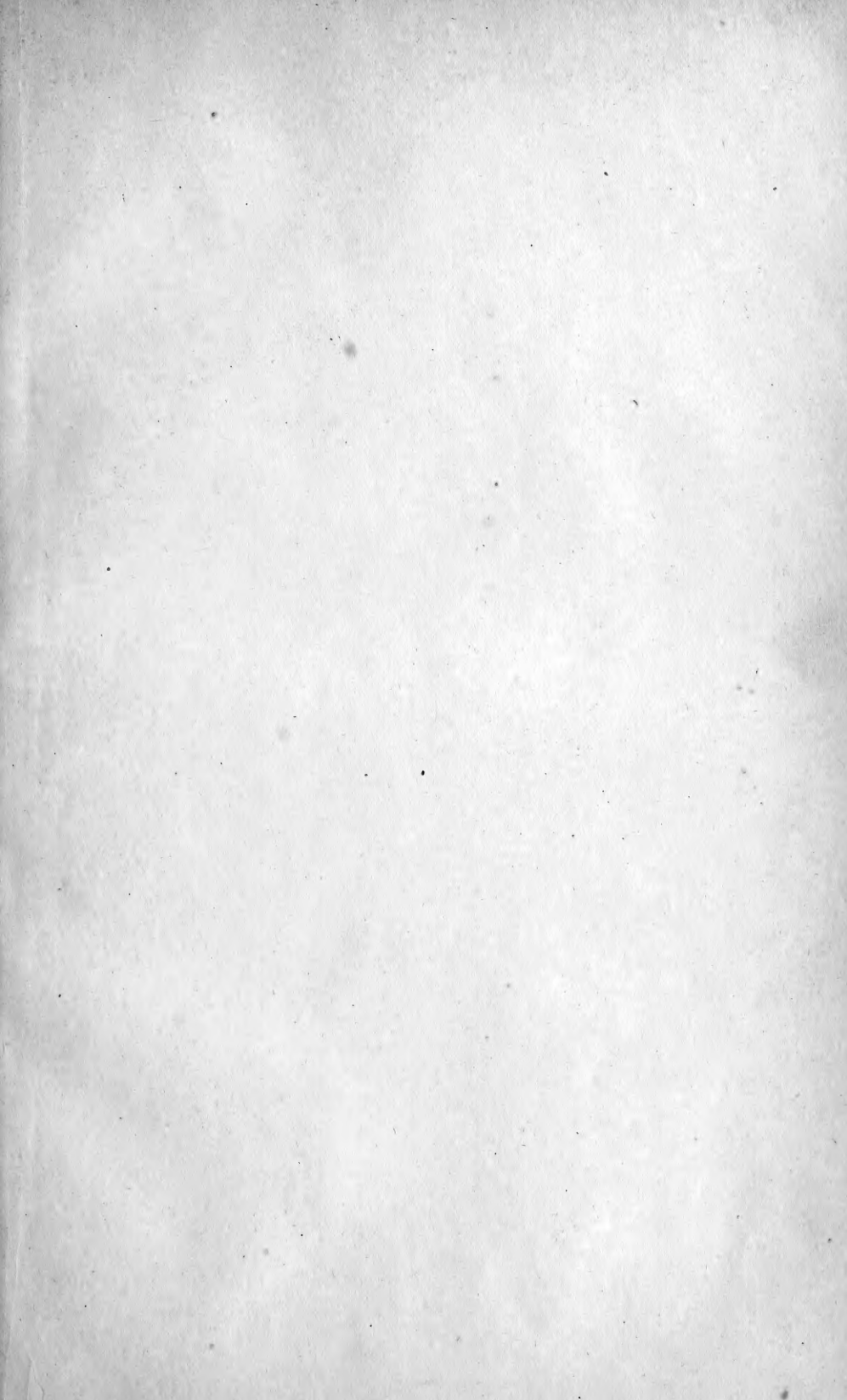
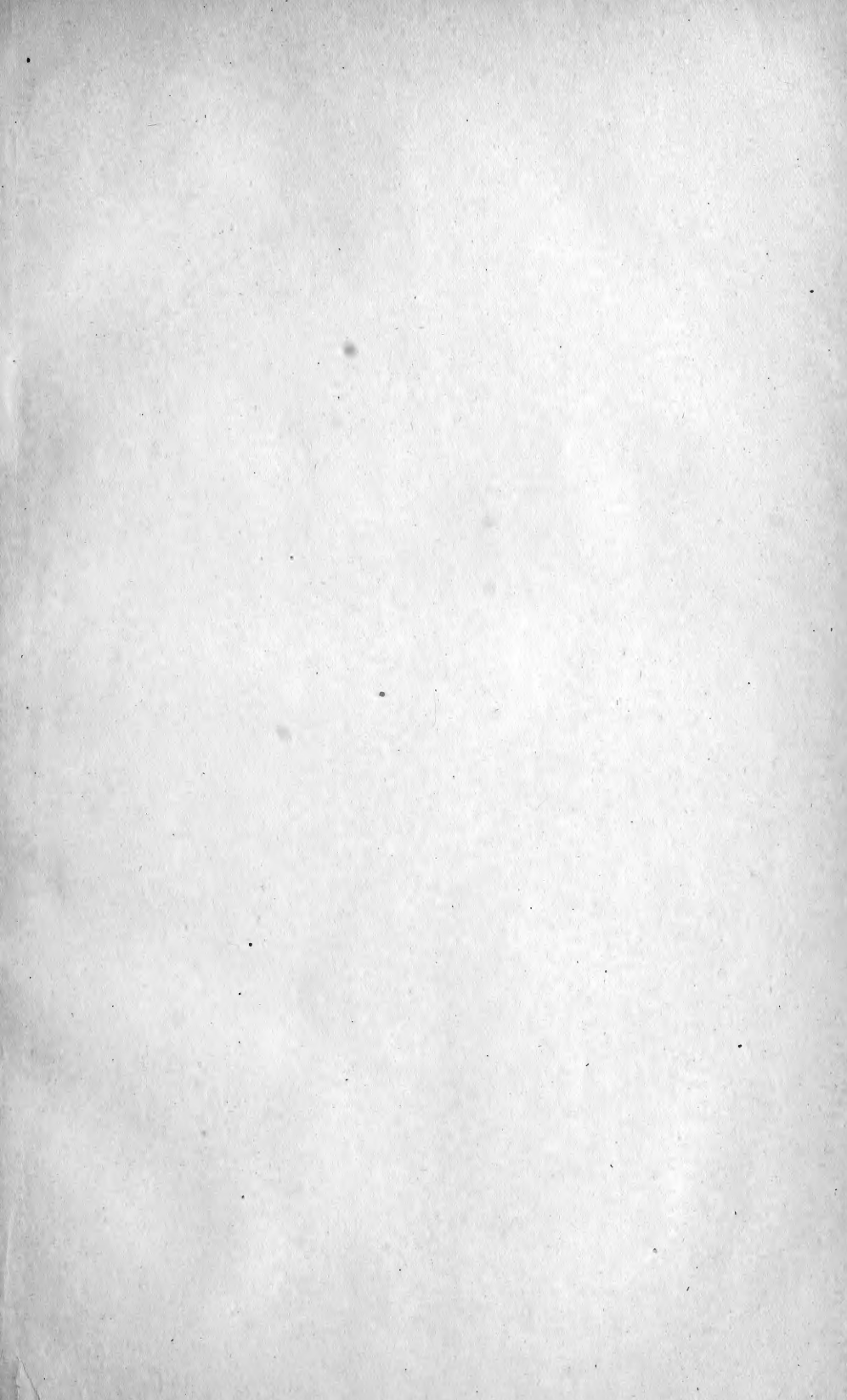


CARDED 1922











THE

MYXOSPORIDIA, OR PSOROSPERMS OF FISHES,

AND THE

EPIDEMICS PRODUCED BY THEM.

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BY

R. R. GURLEY, M. D.,  
*Assistant, U. S. Fish Commission.*

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[Date of publication, December 28, 1894.]



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WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
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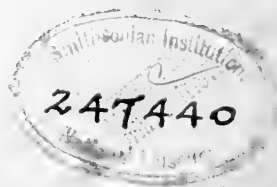
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## 2.—THE MYXOSPORIDIA, OR PSOROSPERMS OF FISHES, AND THE EPIDEMICS PRODUCED BY THEM.

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### INTRODUCTION.

Up to the present time very little attention has been paid to the diseases of fishes, and to their parasites from the standpoint of the effect produced by them upon the host. Yet there can be no doubt that a knowledge of such diseases would be of great practical value. Any one who considers the proportions that fish epidemics may attain will hardly be inclined to question the utility of searching investigation in this direction. Thus, to take a single instance, in the epidemic of 1884 in Lake Mendota, Prof. Forbes<sup>1</sup> states that:

It was estimated that fully 300 tons had died up to that time. On August 7 the Madison Transcript reported that 200 tons had been hauled away by the city authorities during the four weeks preceding and that the fishes were still dying:

Epidemics of similar extent have been reported in Europe.

The important results in the way of prevention of epidemics among domesticated animals and cultivated plants obtained as the result of scientific investigation afford some ground for the hope that similar

<sup>1</sup> Bull. U. S. Fish Com. for 1888 (1890), VIII, p. 482.

results may be obtained here. Obviously the first step in work of this kind is the collection of facts, especially those bearing upon the parasite, its nature, life-history, intermediate hosts, enemies, and its connection (whether causal or otherwise) with diseases or other morbid processes in its host. Such data are a necessary preliminary to preventive or curative measures.

The present paper is a contribution toward the object indicated. A few words now as to its scope. The attempt has been made to compress the entire literature (as far as possible, every known fact) into one article. Further, every form<sup>1</sup> which has been at any time definitely referred to the group is here included. Such collection of forms necessarily involved the exercise of some judgment as to specific identities and distinctions. As most of the known species are available only in the form of descriptions, usually very meager, and of drawings which, especially the older ones, represent only the most general features,<sup>2</sup> it is hardly reasonable to hope that any first attempt at compilation of the synonymy will prove satisfactory in all respects. Still in many cases the synonymy is fairly well established.

The main guide in the correlation of the described forms has been identity of host and seat. Of course it is not contended that this proves, but merely that it more or less strongly suggests, identity of parasite. The confirmatory test is naturally a comparison of figures and descriptions. This latter test will of course be preferred to the test by identity of seat as soon as we shall be in the possession of sufficiently accurate and detailed descriptions and figures, but in the present state of our knowledge the mere absence of difference between more or less incomplete descriptions and figures of two forms with different habitats, produces no conviction in my mind of the identity of the forms. In general it is only where a double correlation (of host and seat on the one side, and of descriptions and drawings on the other) has been possible, that different forms have been united. In other words, the presumption throughout has been in favor of distinctness. From this fact it may be expected that future investigation will tend to reduce somewhat the number of forms here recognized.

The nomenclature has been compared and revised, and for all recognizable species binomial names have been substituted for the clumsy circumlocutions "psorosperms of the pike," etc., formerly in use. It may perhaps be thought that in my preliminary paper and in the present

<sup>1</sup> Although it has been my aim to include in this paper descriptions and figures of all forms ever definitely referred to the *Myxosporidia*, the species noted on pp. 135-137 have been omitted.

<sup>2</sup> It must be further noted that hardly one of the older writers regarded these forms from a taxonomic standpoint. Their principal desire was to work out the life-history and affinities of the group rather than of the individual species; and they seem to have observed the latter mainly for the light they shed upon the life-history of the group as a whole, contenting themselves with designating the different forms as "psorosperms of the pike," etc.

one, too many specific names have been introduced. In answer might be pleaded the difficulty, in a first attempt of this kind, of judging exactly how many species to recognize, and it is not impossible that future experience may require the suppression of a few of the names proposed. Regarding this contingency, however, as one of the incidents of an initial revision, the author will view with considerable equanimity the relegation to synonymy of such names as may prove to be redundant. Finally, as regards this branch of the subject, it should be stated that the main indication seemed to be the building up from the literature of a series of synonymic units which could be later, if necessary, welded into a more compact specific synonymy. This indication has been fulfilled, nearly all the units here constructed consisting merely of an original description and copies of the same by subsequent authors.

The plates appended to this paper include every published figure of every myxosporidian species (species Nos. 27 to 102, inclusive); further, every published figure of every species formerly regarded as myxosporidian but now rejected or queried (species Nos. 1 to 26, inclusive), excepting only some figures of *Psorospermia sciencæ-umbræ*, the figures of the species referred to on pp. 135-137, and the figures of *Lithocystis schneideri* in Schneider's *Tablettes Zoologiques*, which work was not accessible.

In the course of my studies I have been perplexed by the usual number of quotations without any or with only cryptographic references. In the hope of obviating this in the future, intelligible references are given for all statements made and, it is believed, for all important facts.

A number of new terms are introduced in this paper, as it is considered very desirable to have the definiteness and specialization of terms keep pace with the increasing detail of knowledge. They are defined on pp. 120-122. An exceedingly instructive instance of the confusion resulting from the application of the same name to two entirely different structures is afforded by the history of the filaments (see pp. 87-88). If such non-discrimination were to continue far, we should have to construct an elaborate synonymy for every structure as well as for every species.

The lack of a uniform (often, indeed, of any) system of arrangement of data forms, unfortunately, a marked feature in many papers. With very few exceptions the scheme given below has been adhered to throughout this paper. It may not prove to be the best possible, but if it serve to secure the adoption of some regular order (what particular one matters, perhaps, not a great deal) it will have fulfilled its object. The principles underlying it are:

(a) Describe all structures, etc., in the order of their occurrence in the life cycle, beginning with the adult; the process of formation of a structure to precede the description of that structure.

(b) Describe structures in order of position from without inward.

(c) Describe important and constant structures before unimportant and inconstant ones.

(d) Describe structure before function.

The principal exception is the change of place of the cyst, which for convenience is placed before the myxosporidium. Properly (were arrangement an end rather than a means) it should follow the myxosporidium. But the cyst occupies quite a subordinate (almost, so to speak, an accidental) position in the life cycle, and it sheds little light upon any of the structures either of the adult or of the spore. Further, to place it between the myxosporidium and the spore would make an awkward break in the continuity of the life-history.

The following is the order adopted, based upon the principles given:

- |   |  |
|---|--|
| <p><b>I. Synonymy:</b></p> <ol style="list-style-type: none"> <li>a. Recognized binomial name, authority, date.</li> <li>b. Synonymy prior to recognized name, in parenthesis.</li> <li>c. Reference to proposition of recognized name, followed by subsequent synonymy.</li> </ol> <p><b>II. Cyst:</b></p> <ol style="list-style-type: none"> <li>a. Formation.</li> <li>b. Structure.             <ol style="list-style-type: none"> <li>(1) Macroscopic (form, size, color, etc.).</li> <li>(2) Microscopic (a) structure and origin of membrane and (b) contents.</li> </ol> </li> </ol> <p><b>III. Myxosporidium:</b></p> <ol style="list-style-type: none"> <li>a. General characters (form, size, color, etc.).</li> <li>b. Ectoplasm.</li> <li>c. Endoplasm:             <ol style="list-style-type: none"> <li>(1) General description.</li> <li>(2) Nuclei.</li> <li>(3) "Granules" and "globules."</li> <li>(4) Vacuoles.</li> <li>(5) Inclusions, notably pigment.</li> </ol> </li> <li>d. Pseudopodia.</li> <li>e. Amœboid movements.</li> </ol> <p><b>IV. Spore formation:</b></p> <ol style="list-style-type: none"> <li>a. Formation and segmentation of pansporoblast.</li> <li>b. Development of sporoblast into spore (in same order as description of spore, below).</li> </ol> | <p><b>V. Spore:</b></p> <ol style="list-style-type: none"> <li>a. General description (form, size, tailed or not, etc.).</li> <li>b. Shell:             <ol style="list-style-type: none"> <li>(1) Physico-optico-chemical characters.</li> <li>(2) Valves, position and separability.</li> </ol> </li> <li>c. Tail.</li> <li>d. Capsules:             <ol style="list-style-type: none"> <li>(1) Number, position, etc.</li> <li>(2) Filaments.</li> </ol> </li> <li>e. Sporoplasm:             <ol style="list-style-type: none"> <li>(1) Form.</li> <li>(2) Nuclei.</li> <li>(3) Vacuole.</li> <li>(4) "Granules" and "globules."</li> </ol> </li> </ol> <p><b>VI. Exit of sporoplasm, and completion of life cycle with earlier stages of development of myxosporidium.</b></p> <p><b>VII. Habitat; seat, season, frequency.</b></p> <p><b>VIII. Pathological anatomy:</b></p> <ol style="list-style-type: none"> <li>a. Morbid structures (in order of formation):             <ol style="list-style-type: none"> <li>(1) Cell infection.</li> <li>(2) Tumors.</li> <li>(3) Ulcers (later stage of tumors).</li> </ol> </li> </ol> <p><b>IX. Effects and symptoms.</b></p> <p><b>X. Epidemics:</b></p> <ol style="list-style-type: none"> <li>a. Fishes affected; territory covered; extent of ravages.</li> <li>b. Causes.             <ol style="list-style-type: none"> <li>(1) Predisposing or contributory:                 <ol style="list-style-type: none"> <li>(a) Age, etc.</li> <li>(b) Pollution of streams.</li> </ol> </li> <li>(2) Exciting: Mode of infection.</li> </ol> </li> </ol> |
|---|--|

Further, were it not for the abundant evidence to the contrary, furnished by the literature, it would seem superfluous to urge that every report should contain, at least, the following data:

**Host.**—The place and date of collection, the water-temperature, the scientific name<sup>1</sup> of the host, together with the age (or size) of the latter,

<sup>1</sup> Upon this last point too much stress can not be laid. The habit of recording the host merely by the popular name (often local, always more or less ambiguous, and not infrequently designating a whole genus) is greatly to be deprecated, as identification is rendered difficult or impossible, especially for students of other times and countries.



the name of the person collecting, and particularly that of the person identifying it.

*Microscopic technique.*—Especially the fixation process and the stains found most useful should be mentioned.

*Parasite.*—Besides the indications contained in the above outline for arrangement, the gaps in the Tabular Key (pp. 138–165) offer an inviting field for future work. One other point should receive most careful attention, viz, a close comparison of the (at present probably unduly multiplied) forms habitant upon the same host, and especially those in the same organ of the same host. In this way a few years will suffice to condense the present synonymy to its proper dimensions. It may be added that even the dimensions of the spores—the most accurate of all data—are sometimes omitted.

*Effects and epidemics.*—Above all, attention should be directed to gathering accurate data as to the extent, the species of fishes affected and those exempt, the territory invaded, the season, as far as possible the relative potency as causative factors of temperature, water pollution, etc. The effects of all remedies tried, whether successful or not, should of course be recorded.

*Reduction of measurements.*—The older authors recorded their measurements in thousandths of a line.<sup>1</sup> I have reduced these to  $\mu$ 's. Owing to the number of inches (also, consequently, of lines) in use in Germany, the original measurements are quoted in parenthesis. In 1853 Robin<sup>2</sup> reduced the German measurements to decimals of a millimeter. He assumed  $1''' = 2.25$  mm. Bütschli<sup>3</sup> adopts the same equivalent for the "*Linie*" ( $'''$ ). Wherever my results differ from Robin's I have noted his figures in parenthesis along with the original measurements.

The following are the calculations and the resulting equivalents adopted:

One Prussian foot = 1.0298 English feet.

One Prussian inch = 1.0298 English inches.

One m. = 39.371 English inches = 38.2317 Prussian inches.

One mm. = 0.0382317 Prussian inches = 0.45878 Prussian lines.

Thus 1 "*Linie*" = 2.18 mm. nearly instead of 2.25 mm.

Fortunately the discrepancies are slight. All spore-measurements are in  $\mu$ 's; cyst measurements in decimals of a millimeter.

As regards the translations, I am responsible for all, with the exception of Kolesnikoff's article the translation of which was made by Mr. Israeli, of the Surgeon-General's Library. Dr. Robert Stein, of

<sup>1</sup> In the only case where I could find a direct comparison between Müller's "*Linie*" and the millimeter, viz, Müller's translation of Gluge's  $\frac{\pi}{100}$  of a mm. for *Glugea anomala* (Gluge, Bull. Acad. Roy. Belg., 1838, v, p. 774; Müller, Müller's Archiv., 1841, p. 491), as 0.0020''', Müller regards the former as equal to 2 mm.

<sup>2</sup> Hist. Nat. des Végét. Parasites.

<sup>3</sup> See *Chloromyxum mucronatum* (p. 264).

the U. S. Geological Survey, has, however, helped me in a number of points connected with this branch of the subject.

I am indebted to many friends for assistance. In particular, I wish to acknowledge my deep indebtedness to Dr. C. W. Stiles, of the Department of Agriculture, for numerous judicious suggestions and for encouragement and aid in very many ways, especially in the study of the nuclei. M. Thélohan very generously placed at my disposal notes on the synonymy of several species. The synonymy of the piscine hosts has been revised by Dr. Theodore Gill. Finally, I desire to thank the officials connected with the Library of the Surgeon-General, U. S. Army, for numerous courtesies extended me in the course of a protracted examination of the valuable collections under their charge.

As far as possible, this paper has been brought up to January 1, 1894. Several subsequent papers have also been included (see pp. 128-129).

## GENERAL DESCRIPTION OF THE SUBCLASS MYXOSPORIDIA.

## I.—NOMENCLATURE AND DEFINITION.

## SUBKINGDOM PROTOZOA.

## Class SPOROZOA Leuckart, 1879 (emended).

The following is Leuckart's definition<sup>1</sup> verbatim, with the exception of the proposition of the *Gregarinida* as the type order, a proposition that is implied by Leuckart's language. The words inclosed in brackets should, as shown by subsequent observations, be omitted from the class definition.

Unicellular parasites [of stable body-form], destitute [of pseudopodia and] of ciliæ, covered with a smooth, more or less solid cuticle. At the anterior end not seldom a probosciform attachment-apparatus. Movements on the whole little striking, worm-like or feebly amœboid. Mode of life always parasitic; nutrition by endosmosis. Reproduction by more or less hard-shelled spores (pseudonavicellæ; psorosperms) formed in the interior of the protoplasm in variable but very considerable numbers,<sup>2</sup> either progressively or simultaneously (in the latter case at the termination of growth and after encystment). Germinal portion of spore consisting of falciform protoplasmic rods (*Gregarinida*; *Coccidia*) or a single protoplasmic mass (*Myxosporidia*); type order *Gregarinida*.

## Subclass MYXOSPORIDIA Bütschli, 1881.

Zoolog. Jahres-Ber. f. d. J. 1880, I, p. 162; *ib.*, Bütschli, 1881, Ztschr. f. wiss. Zool., XXXV, pp. 630, 650; *ib.*, Bütschli, 1882, Bronn's Thier-Reich, I, p. 590; *ib.* of all subsequent authors; *Myxosporidæ* (Psorospermidæ J. Müller)<sup>3</sup> Zürn, 1882, Die thierischen Parasiten, Weimar, p. 816; *Myxospora*<sup>4</sup> (error) Mégnin, 1885, Compt. Rend. hebdom. Soc. Biol. Paris, II, p. 447; subclass *Myxosporidia*, Lankester, 1885, Encycl. Britan., 9 ed., XIX, p. 855; "Psorospermidæ J. Müller,"<sup>5</sup> Koch, 1887, Encyklop. d. gesamt. Thierheilkde u. Thierzucht, IV, p. 94.

## THE SUBCLASSIC DESIGNATION.

Müller, in 1841, denominated the forms observed by him merely as "Psorospermien." Everything points to the conclusion that this name was used merely indefinitely as a group designation. He neither proposed it as a generic name nor did he anywhere latinize it. He

<sup>1</sup> Die Parasiten des Menschen, 1879, 2 ed., p. 241.

<sup>2</sup> Compare *Bisporogenesis* in index.

<sup>3</sup> An error; Müller did not propose any such family. Zürn's definition is quoted to show the errors (*italics*):

"Order 4. *Myxosporidæ* (*Psorospermidæ*, J. Müller). Frequent in and on fishes and Amphibia. The nucleus-less, often granulated protoplasm, is surrounded tube-like by a cuticle. From the young protoplasm of these tubes, single or double contoured, fusiform, oval, or round spores originate *without previous encystment*. In the spore originate one or several germs, mostly resembling a nucleus-less, but somewhat granulated plasma-globule, or representing a needle-shaped (*stabförmige*) body. The spore membrane often provided with 1 or 2 filaments) bursts in order to free the only very rarely motile germs."

<sup>4</sup> "Psorospermies des poissons ou *Myxospora* de Bütschli."

used it in the same sense in the paper published by him and Retzius in 1842, and was followed in this use by Creplin, also in 1842. In 1843 his article of 1841 was reprinted in French in Rayer's Archives. In this the German "Psorospermien" is rendered by the French "psorospermies," both of them the exact equivalent of the general indefinite English plural *psorosperms*. If anything is needed to complete the evidence it is found in the fact that not one of these observers proposed a single binomial name. So it is certain that the term was used by Müller and his immediate successors as a general group term and not as a generic designation. And it was so used in 1845 by Dujardin, and in 1851 by Leydig, neither of whom employed a generic name. Further, they did not use any specific (binomial) names, all of their species, like those of previous authors, being designated as "psorospermies du brochet," "Psorospermien der Hecht," or by a similar title.

The first author to apply the binomial nomenclature to the "psorosperms" was Charles Robin. In his *Histoire Naturelle des Végétaux Parasites* (1853) were collected descriptions and figures of nearly all of the previously described forms. Robin there defines the "psorosperms" as a tribe of Diatoms, as follows:

Tribus Psorospermæ Ch. R.

Phycoma ex cellulis organicis compositum; cellulæ albæ, fuscæ, lutescentes vel achromaticæ. Generatio ignota. (Piscium parasiticæ.)

I form this group to receive a certain number of species of parasitic forms described first by J. Müller, and since carefully studied by him, Retzius, and myself.

From the foregoing it will be seen that to the subclassic (ordinal or tribal) name was appended an exceptionally clear definition. In the group thus defined Robin placed a single genus, *Psorospermia* Robin, which must, therefore, stand as the type genus of the group. His generic definition was: "*Characteres tribus.*" Robin failed to designate any particular species as the generic type. He reproduced descriptions and figures of 10 forms made known by other authors, under the customary headings of "psorosperms of the pike," etc. In addition to this, however, he inserted a description and figures of a single species of his own, which was the only one provided with a binomial name, or in other words the only species (in the nomenclatural sense) present. It is plain, therefore, that this species (*P. sciænæ-umbræ* Robin) must stand as the generic type.<sup>1</sup>

Curiously enough, however, of all the species collected by Robin this is almost the only one which can not be regarded as a myxosporidian. That it can not be so regarded is evident from a careful examination of his definition and figures. Unfortunate as it is that the name *Psorospermia* must henceforth be restricted to organisms having

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<sup>1</sup>In order to place the matter beyond doubt, I now propose to limit the genus *Psorospermia* Robin, as above indicated, viz: to forms of the type of *P. sciænæ-umbræ* Robin, which species I propose as the generic type. I further propose *Psorospermia* as the type genus of Robin's tribe *Psorospermæ*.

no affinity with the "psorosperms," it is none the less inevitable that, as between the generic definition and the type species, the generic name must follow the fate of the type species.

Robin's name *Psorospermia* can not, therefore, be employed as the sub-classic designation of, and for the same reason it can not be used as a generic name in, the *Myxosporidia*.

In this connection it may be noted that the name *Psorospermium* has obtained currency in the *Sporozoa*. Apparently I have not found the original use of the name, and can only give the following references. The forms are nonmyxosporidian (see also p. 135)-

*Psorospermium*, Paulieki, 1872, Mag. f. d. gesamm. Thierheilkde, Berlin, XXXVIII, p. 6; *ib.*, Rivolta, 1878, Giorn. Anat. e Patol., Pisa, X, p. 233.

#### THE SUBCLASSIC DEFINITION.<sup>1</sup>

*Sporozoa*, whose adult stage is characterized by the presence of numerous nuclei originating by division; further by the power of amœboid movement,<sup>2</sup> and by the mode of spore formation, which takes place in definite transparent areas (pausporoblasts), and which is progressive, not being confined to the last stage of the life cycle;<sup>3</sup> whose spores exhibit always 2 and sometimes 3 axes of symmetry and possess a shell resistant to chemical reagents, 1 or more capsules (each inclosing a coiled filament capable of extrusion), and a single mass of sporoplasm; type order *Phanocytes*.

## II.—MORPHOLOGY.

### GENERAL DESCRIPTION OF STRUCTURE.

Omitting discussion of controverted questions and of peculiarities correlated with generic differences, the life-history and morphology of the subclass may be briefly outlined as follows:

In all the *Myxosporidia* two distinct stages are recognizable, viz, the myxosporidium (growth-reproduction or adult stage) and the spore. In addition a cyst may be present (see p. 77).

1. *The myxosporidium*.—At the time of its exit from the spore the myxosporidium possesses nuclei and sometimes a vacuole. It now<sup>4</sup>

<sup>1</sup> Original. The first definition of the group was given by Lankester, as follows:

"Sporozoa, in which the euglena-phase is a large multinucleate amœba-like organism. The cysts are imperfectly known, but appear to be simple. Some attain a diameter of two lines. The spores are highly characteristic, having each a thick coat which is usually provided with a bifurcate process or may have thread capsules (like nematocysts) in its substance. The spores contain a single nucleus and are not known to produce falciform young, but in one case have been seen to liberate an amœbula. The further development is unknown. The *Myxosporidia* are parasitic beneath the epidermis of the gills and fins, and in the gall bladder and urinary bladder of fishes, both fresh-water and marine."

<sup>2</sup> Except possibly *Thelohania*, in which the myxosporidium is unknown.

<sup>3</sup> Noted by Bütschli (Bronn's Thier-Reich, 1882, I, p. 595) in *Myxobolus mülleri* and *Myxidium lieberkühni*.

<sup>4</sup> Fide Pfeiffer; cf. Korotneff; see pp. 187, 288, pl. 9, fig. 1, and pl. 46, fig. 1b.

enters upon an intracellular existence, penetrating into the interior of the red blood and other cells of the host, where, protected by the cell membrane, it grows by feeding on the cell contents. Finally, its continued growth produces distension, and ultimately rupture of the cell-membrane, and the myxosporidium becomes free. It now moves about amœboidly, grows larger, the nuclei become more numerous through karyokinesis, and spore formation begins. This last process is not confined to the last stages of the life cycle, but begins early and is progressive.

At this period the myxosporidium exhibits the following structure:

In outline it is vermiform, sacculated, roundish or not infrequently entirely irregular (see pls. 29, 37-39, 43-45). It usually possesses the power of amœboid movement and generally exhibits a distinct separation of ectoplasm and endoplasm (see pl. 39, figs. 1, 2, and pl. 44, fig. 1).

The ectoplasm (see pl. 16, fig. 4; pl. 39, fig. 1; and pl. 44, fig. 3) is very transparent, quite or nearly destitute of granules, sometimes more or less radiate-striate, and is often prolonged into pseudopodia which only involve the endoplasm when of very large size. The pseudopodia sometimes form a shaggy or bristly coat over the whole, or a part of the myxosporidium (see pl. 44, fig. 1a).

The endoplasm (see pl. 37, fig. 4; pl. 38, fig. 1, and pl. 39, fig. 1) is more or less coarsely granular and contains numerous nuclei, fat-globules, hæmatoidin crystals (pl. 44, fig. 5) and other pigment. The nuclei are derived from the primitive nuclei of the myxosporidium (the nuclei of the sporoplasm; see below). The hæmatoidin crystals are usually found within the fat-globules. The myxosporidium may contain other extraneous pigment, e. g., bile-, and perhaps a proper, pigment (see pp. 76, 277).

*Spore formation:* Each nucleus attracts to it a portion of the surrounding myxoplasm to form a pale, solid globule termed the pansporoblast (pl. 12, fig. 1 *a-c*, and pl. 47, fig. 1 *a, b*) which later segments into a number of sporoblasts (pl. 12, fig. 1 *h, i*, and pl. 47, fig. 1 *c, d*), each of which develops into a spore.

2. *Spore.*—This always contains three elements, shell, capsule with filament, and sporoplasm. The shell (see pl. 16, fig. 3, and pl. 28, fig. 1) is exceedingly transparent, very resistant to chemical reagents, and is frequently bivalve. The capsule (pl. 26, fig. 7, *cap.*) is a pyriform, hollow, elastic-walled body which always contains a single coiled thread (*filament*) capable of extrusion. The sporoplasm (pl. 26, fig. 7, *spo.*) is always a single mass of protoplasm. It contains nuclei (*n*), and sometimes a vacuole (fig. 7, *vac.*), which when present is always single. At maturity the shell splits when bivalve, or dissolves when univalve, thus setting free the sporoplasm (pl. 15, fig. 7 *b*), which, now become the myxosporidium, rebegins the life cycle.



## DETAILED DESCRIPTION OF INDIVIDUAL STRUCTURES.

## "PSOROSPERMS" THE SPORES.

The older writers seem to have tacitly admitted that their "psorosperms" represented the spore stage. Thus Lieberkühn<sup>1</sup> says that certain animals fix themselves to the skin of fishes and in reproduction fall apart into the "psorosperms." Balbiani,<sup>2</sup> however, regarded the "psorosperms" as an adult cryptogam. This view he subsequently virtually abandoned.<sup>3</sup> All the later authors, without exception, have regarded the myxosporidium as the adult.

## THE MYXOSPORIDIUM.

This was first observed by Dujardin in 1845 (see p. 273). It occurs free or attached. Size 2 mm. or, more usually, much less, without constant or characteristic body-form, being cylindrical, ribbon-, or club-shaped, or more or less globular or irregularly amœboid, consisting of colorless or more or less yellowish protoplasm (pigment usually extraneous, see p. 76); usually, probably always, showing a more or less (frequently quite) distinct differentiation into ectoplasm and endoplasm. In the cyst-forming *Myxosporidia* (e. g., the branchicolous forms) the differentiation is also, at least in the older myxosporidia, very sharp.

## ECTOPLASM.

Forming a very transparent granule-free or exceedingly finely granular zone, from which all of the elements characteristic of the endoplasm are absent.

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<sup>1</sup> Müller's Archiv., 1854, p. 357.

<sup>2</sup> Compt. Rend. Acad. Sci. Paris, 1863, LVII, p. 159.

<sup>3</sup> Journ. de Microgr., 1883, VII, pp. 198, 201, 276.

<sup>4</sup> Pfeiffer regards the large myxosporidia as composed by the fusion of many small ones. He thus explains progressive spore formation:

"With the view here expressed that the smallest psorosperm-tubes of the barbel are simple myxosporidia ('sporoblasts') similar to those of *Eimeria* in the schematic table, and to those of the *Microsporidia*; further, that the large tubes are a conglomerate of many different individual parasites which have run together accidentally in Gregarine fashion, and that their cyst nature originates through cicatricial incapsuling by the host, some things apparently do not entirely agree. Why are the large tubes empty in the middle? Where have the contents gone? (They can not be a consumed residual mass.) How are to be explained the appearances simulating nuclear division on the capsule wall in figs. 9 and 14? Does this last-mentioned fact compel us to admit after all a progressive endogenous division and a successive infection? We have above answered this in the negative; they must admit of definite solution when more comparative investigations (e. g., upon batrachians and birds) shall be at hand."

Subsequently (see p. 227) he explains the emptiness of the central portion by a supposition of spore-migration towards the periphery in search of better nutritive conditions.

A similar pressure-fusion occurs in "*Myxosporidium*" *bryozoides* (p. 188).

## ENDOPLASM.

Much more coarsely and more or less yellowish granular, containing numerous nuclei, fat-globules, and sometimes one or more vacuoles; also pigment.

*Nuclei*.—First observed by Prof. Bütschli<sup>1</sup> in *Myxidium lieberkühnii*, where their nuclear nature was shown both by their structure and by their affinity for carmine; always very numerous, the smallest occurring only in the youngest forms, strewn irregularly through the endoplasm. As in a number of species the nuclei have been observed to originate by division, there is every reason to suppose that such origin obtains throughout the subclass<sup>2</sup>, and that the myxosporidium nuclei are to be referred back to the nuclei of the sporoplasm.

*"Granules" and "globules."*—Many of the structures loosely termed "granules" and "globules" by the older authors are really nuclei, and this should be borne in mind in reading their descriptions, which have sometimes been reproduced without change (see also pp. 209, 220).

According to Bütschli (see page 285), these bodies are of a fatty nature, as shown by their complete solubility in alcohol. According to several other authors, the hæmatoidin crystals are found within globules whose fatty nature was presumed from the same reaction. Thélohan, however (see below), while admitting the solvent action of alcohol upon certain chromatophorous globules observed by him in *Chloromyxum leydigii* and in *Myxidium lieberkühnii*, denies their fatty nature, as osmic acid is without action upon them.

*Fat-globules*.—Feebly glittering; size variable; always present except in very young individuals; especially frequent in *Myxidium lieberkühnii*.

*Vacuoles*.—Sometimes one or more; number, position, and presence inconstant; apparently always nonpulsating.

*Pigment*.—Although it has heretofore seemed probable<sup>3</sup> that all pigment occurring in the *Myxosporidia* was of extraneous origin, it would appear now, from Thélohan's recent observations, as though perhaps the presence of proper pigment must be admitted. This observer says:<sup>2</sup>

In many myxosporidia which live in the free state in the natural cavities one finds the endoplasm riddled with strongly colored globules whose tint varies from golden yellow to brown. Very numerous in *Myxidium*, they give to the internal face of the pike's bladder a characteristic yellow tint; they also exist in *Chloromyxum leydigii* (Mingaz.). As these elements do not resist the action of alcohol or that of the essential oils, one finds no trace of them in sections; they are not fatty, as osmic acid is without action upon them.

*Chloromyxum fluviatile* also contains similar structures.

<sup>1</sup> Ztschr. f. wiss. Zool., 1881, xxxv, pp. 632-633; Bronn's Thier-Reich, 1882, I, pp. 594-595. Bütschli (1882) was the first to suggest the generality in the *Myxosporidia* of the multinucleate condition. Lankester (see p. 73, foot note 1) took the same view.

<sup>2</sup> This is also Thélohan's opinion (Bull. Soc. philomat. Paris, 1892, IV, p. 169).

<sup>3</sup> As Bütschli remarked in 1881 (Ztschr. f. wiss. Zool., xxxv, pp. 642, 649). Cf. also *Pigment in index*.

The extraneous pigment consists of hæmatoidin crystals, whose origin, mode of occurrence, etc., are discussed elsewhere (p. 285).

*Pseudopodia*.<sup>1</sup>—Usually blunt, simple or lobed ectoplasmic processes, involving the endoplasm only when very large. In *Myxidium lieberkühni* subpermanent bristle-like pseudopodia have also been observed (see p. 285).

*Amœboid movements*.—These have been seen in a number of species.<sup>2</sup> They are slow or active; sometimes absent, owing to the deleterious effect of so-called "indifferent" fluids.

#### THE CYST.

*Encystment*.<sup>3</sup>—This—or at least the tissue-imbedding which is so termed (see below)—is the usual preliminary to reproduction in *Myxobolus*. Reproduction takes place without it, however, exceptionally in *Myxobolus*, and constantly in those forms inhabiting the cavities of the hollow organs.<sup>4</sup>

#### MACROSCOPIC APPEARANCES.

The most striking feature of the myxosporidian cyst is the *invariable absence of pigmentation*. It is always of a cream-white color.<sup>5</sup> In size it varies within very wide limits, from a fraction of a millimeter to clusters of several centimeters in length. Shape also extremely variable, mostly spherical to fusiform. Usually it is easily detachable from its place in the tissues. The cyst contents are always milky or creamy, usually fluid, sometimes from deficiency of water, caseous, and consist of spores and more or less "granular matter."

#### MICROSCOPIC APPEARANCES.

*Cyst membrane*.—In harmony with his view of the nature of the contents of the *Glugea anomala* cyst, Gluge<sup>6</sup> regarded the cyst membrane as formed by the "solidification of an albuminous matter" of the host.

Concerning this structure in *Myxobolus mülleri*, Bütschli<sup>7</sup> remarks that it differs from the type of membrane usual among the unicellular organisms (particularly the Gregarines) in its plasmatic nature, being

<sup>1</sup> In Mlle. Leclercq's description of the *Myxosporidia* (Bull. Soc. Belg. de Microsc., 1890, xvi, p. 100) the erroneous statement is made that the *Myxosporidia* do not emit pseudopodia.

<sup>2</sup> Notably *Myxobolus ellipsoides* and *Myxidium lieberkühni* (pp. 222, 286).

<sup>3</sup> From the view that the *Myxosporidia* undergo a true (zoological) reproduction-encystment, Bütschli (Bronn's Thier-Reich, 1882, i, pp. 592, 593) dissents.

<sup>4</sup> Cf. Lieberkühn, 1854, Bull. Acad. Roy. Belg., xxi, pt. 2, p. 23; Thélohan, 1890, Annal. de Microgr., ii, pp. 197-198.

<sup>5</sup> Of course not all white (nonpigmented) cysts are myxosporidian. Some Trematodes occur in similar cysts, though they seem more usually to excite the deposition of pigment.

<sup>6</sup> Bull. Acad. Roy. Belg., 1838, v, p. 775.

<sup>7</sup> Ztschr. f. wiss. Zool., 1881, xxxv, pp. 632, 633; Bronn's Thier-Reich, 1882, i, pp. 592, 593.

composed of clear, very finely granular protoplasm, containing many small nuclei which possess a distinct dark membrane and a somewhat irregular outline, and stain intensely with alum carmine. It is difficult to determine certainly whether this membrane is formed by the myxosporidium or by the host. Opposing the myxosporidian origin (which, however, is in no wise excluded) is the relatively greater size of the membrane nuclei compared with those of the endoplasm.

Balbani's<sup>1</sup> views of cyst structure may be summed up thus:

Membrane of rather firm texture, very thick (sometimes  $10\ \mu$ ) without structure, showing small refringent granulations. In spite of Bütschli's assertion of the presence of carmine-staining nuclei, Balbani could find nothing definite. He is disposed to regard the membrane as a production of the parasite rather than of the host.

Ludwig<sup>2</sup> believes the cyst membrane to be probably a production of the host.

Thélohan<sup>3</sup> could find no nuclei in the cyst membrane and believes their absence an argument of real value in favor of the derivation of the membrane from the (similarly nonnucleated) myxosporidian ectoplasm. Finally, he says, *Cystodiscus immersus* (which is free-floating) is surrounded by a clearly defined structureless membrane.

Perugia<sup>4</sup> has, it seems to me, recently made an important contribution to this subject. This observer has seen in *Myxobolus mugilis* a cyst which contained *three separate myxosporidia*. (See p. 213, pl. 14, fig. 5.) It is hard to resist the conclusion that, in this case at least, the host furnished the cyst membrane. But it is equally difficult to deny that in certain other forms, especially *Cystodiscus immersus*, which is free-floating in the bile, (1) that there is a membrane and (2) that such membrane is a product of the myxosporidium. Still other species (e. g., *Myxidium lieberkühni*) show an ectoplasmic membrane. I suspect the explanation to be that the "cyst membrane" is really composed of two concentric membranes, one (the inner and constant one, whose degree of development and of condensation, however, probably varies greatly) being the ectoplasm of the myxosporidium and the other (the outer and inconstant one, being absent, for example, in the free-floating forms) being a product of the tissues of the host.

Finally Thélohan<sup>5</sup> has recently put forth essentially the same view, viz, that the so-called cyst membrane is not derived from but is merely the ectoplasm of the myxosporidium modified. His observations are as follows:

Those *Myxosporidia* which form well-defined cysts (e. g., the branchiocolous species) have the ectoplasm still distinct, but no pseudopodia are seen. Formerly he admitted the existence of a cyst membrane

<sup>1</sup> Journ. de Microgr., 1883, VII, pp. 199, 200.

<sup>2</sup> Jahresber. d. rhein. Fisch.-Vereins Bonn, 1888, p. 31.

<sup>3</sup> Annal. de Microgr., 1890, II, pp. 203-205.

<sup>4</sup> Boll. Scientif., Pavia, 1891, XIII, pp. 23, 24.

<sup>5</sup> Bull. Soc. philomat. Paris, 1892, IV, pp. 168, 169.

formed by the parasite. Thélohan now believes a true membrane to be absent, the pseudo-membrane being merely the denser, most external layer of the ectoplasm, peculiarly modified (coagulated and contracted) under the action of the fixing and hardening reagents. It can then take on the aspect of a membrane, the resemblance being sometimes even further heightened by its exhibiting very definite striæ.

Sections of a barbel's intestine showed connective tissue spaces each inclosing a myxosporidium with an often very well differentiated external zone which presented a very distinct striation. Although at first regarding this as a confirmation, Thélohan, after a more thorough examination, varying the observation methods and studying a great number of sections of different myxosporidian species, became convinced that these pseudo-membranes are artificial productions, the result of a rougher action of the reagents on the more exposed external ectoplasmic layers, which action accentuates their differentiation and exaggerates their characters. In fact this membraniform layer can be seen to become continuous, without a line of demarcation, with the ectoplasm proper.

Further, a similar appearance was sometimes observed in sections of the pike's urinary bladder, where (the myxosporidia being free and motile) there can be no question of a cyst membrane. Moreover, the distinction is much more apparent in sections after the action of reagents (under which conditions the limit of the 2 layers is clearly indicated and marked by a continuous, often very pronounced, line) than in fresh preparations.

Thélohan<sup>1</sup> says that, as Bütschli remarks, the age of the cyst can be inferred from its size, the less advanced cysts being larger with a central zone containing the older spores and an outer one containing nuclei and spores in process of formation. The oldest cysts are small, contain no nuclei, and spore formation has ceased, only developed spores being present.

## SPORE FORMATION

### GENERIC RELATIONS.

This process exhibits differences which not only serve as ordinal characters, but which appear also to stand in some sort of relation to generic lines.

Thus in *Glugea* we have polysporogenetic pansporoblastic spore formation within a myxosporidium, the pansporoblast not subsistent as a sporophorous vesicle.

In *Pleistophora* we have polysporogenetic pansporoblastic spore formation, no myxosporidium (completely transformed into pansporoblasts?), the pansporoblast subsistent as a sporophorous vesicle.

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<sup>1</sup> Annal. de Microgr., 1890, II, p. 204.

In *Thelohania* the myxosporidium appears to be absent (completely transformed into pansporoblasts?); the pansporoblast constantly produces 8 spores.

The process in *Cystodiscus* is imperfectly known (see p. 280).

Nothing is known of the process in *Sphaeromyxa*.

The rule in *Myxobolus* appears to be pansporoblastic spore formation with tripartite sporoblast segmentation. Although at first sight *M. mülleri* appears to constitute an exception to the rule, I have endeavored elsewhere (p. 218) to show that this species really conforms to it.

*Chloromyxum* (as represented by *C. leydigii*; also *C. incisum*) throughout all its various habitats is characterized by monosporogenous pansporoblastic spore formation. In *C. mucronatum*, however, Lieberkühn appears to have observed 2 spores in the pansporoblast.

Nothing is known of the process in *Sphaerospora*.

In *Myxosoma* also nothing is known beyond the fact that the spores are developed within a myxosporidium.

Beyond the very striking peculiarity of bisporogenesis, nothing is known as to the process in *Ceratomyxa* (see p. 274).

*Myxidium* (*M. lieberkühnii*) appears to be characterized by pansporoblastic spore formation, without sporoblast segmentation. As, however, in *M. lieberkühnii* the developed capsule is a structure plainly separate from, and not continuous in substance with, the sporoplasm, its abstriction from the latter must occur at some period of the development. As this abstriction would differ from the *Myxobolus* segmentation mainly in the time of its occurrence, the real amount of difference between the 2 processes becomes problematical.<sup>1</sup>

#### HISTORY.

From the following (which, unfortunately, I have been unable to examine further) it seems to me probable that Leuckart recognized the pansporoblast as early as 1847. In speaking of the spores, he says:<sup>2</sup>

Their formation takes place in an endogenous manner in the interior of special cells, as I have already shown in another place (Göttingische Gelehrte Anzeiger, 1847, p. 1032).

Leydig's description<sup>3</sup> is as follows:

A clear pale-contoured vesicle is first differentiated, in which a number

<sup>1</sup>Prof. Bütschli (Bronn's Thier-Reich, 1882, I, p. 600) takes, apparently with special reference to this species, the view that the capsules seem to lie not near, but in the sporoplasm, which appears to cover them with a delicate prolongation. This view is also, he remarks, to be expected from the developmental history. This, however, doubtless means only that the capsules are surrounded on all sides by the sporoplasm, not that they are continuous in substance therewith.

<sup>2</sup>Archiv. f. physiol. Heilkde, 1852, XI, p. 435.

<sup>3</sup>Muller's Archiv., 1851, p. 226. Leydig, it will be remembered, erroneously regarded this structure as a vesicle (*Tochterblase*). His observations were made upon *Chloromyxum leydigii* and *C. incisum*.



of granules originate. During the subsequent progress in development up to the ripe psorosperm, changes take place in the form of the vesicles, the character of the contour, and the contained corpuscles. The latter first elongate, one pole becomes sharpened, the whole corpuscle assumes the familiar clearness of outline, the granules diminish in number and form (perhaps through fusion or after previous solution) the 4 capsules. The contour of the sporoblast also becomes sharp.

Lieberkühn (see *Chloromyxum mucronatum*, p. 265) first noted the pansporoblast as a solid plasma-sphere, but he did not trace the connection of the solid sphere with Leydig's vesicles.

In 1880, Gabriel noted, in *Myxidium lieberkühnii* (see p. 287), that the vacuole stage of the pansporoblast is a subsequent and not the original condition. It is quite evident, however, that he did not understand the mode of pansporoblast formation.

In 1881, Bütschli<sup>1</sup> showed that the pansporoblast is primarily not a vacuole, but a plasma-sphere. The segmentation of this and the development of the resulting sporoblasts were also traced.

#### PROCESS.<sup>2</sup>

*Formation and segmentation of the pansporoblast.*—The first step in pansporoblast formation is the condensation around each of the numerous nuclei (of the endoplasm) of a small clear-contoured sphere of myxoplasm, which seems limited by a thin envelope resulting from a condensation of its peripheral layer, the whole constituting a pansporoblast. This subsequently shrinks slightly, so as to appear as a ball surrounded by a vacant space, and this latter in its turn by the membrane. The nucleus then divides (by karyokinesis) and redivides so that one very soon has a sphere (pansporoblast) with a dozen nuclei. The sphere then segments into two hemispheres (sporoblasts) which remain surrounded by the original pansporoblast membrane. Each sporoblast contains several nuclei (see below). The nuclei which do not enter into the formation of the two sporoblasts are rejected and are found in a small mass of protoplasm which remains (along with the two sporoblasts) within the original pansporoblast membrane.

In this connection it is well to quote from Kunstler and Pitres<sup>3</sup> the following erroneous description:

This envelope [the ectoplasm] would contain, according to Bütschli, small nuclei. The nuclei, in proportion as the cyst [membraned myxosporidium] enlarges, divide; the protoplasm is condensed around them to form oval bodies, which Balbiani considers the spores; this author has indeed seen there the formation of four falciform corpuscles [*italics my own, for errors*].

<sup>1</sup> Ztschr. f. wiss. Zool., xxxv, pp. 645-646; Bronn's Thier-Reich, 1882, I, p. 596.

<sup>2</sup> Description based upon Thélohan's (Compt. Rend. Acad. Sci. Paris, 1890, cxi, p. 693). For the process in the *Cryptocystes*, see p. 201.

<sup>3</sup> Journ. de Microgr., 1884, viii, p. 474.

*Development of the sporoblasts into the spore.*—As noted by Bütschli and Balbiani<sup>1</sup> in the 2-capsuled forms (*Myxobolus*), each sporoblast divides into 3 unequal uninucleated masses, 2 small and 1 large, destined to form respectively the 2 capsules<sup>2</sup> and the sporoplasm.

*a. Development of the capsules.*—Very soon there is produced in each of the two smaller masses, ordinarily in the neighborhood of the nucleus (see above) a small, rounded, clear vacuole, distinguishable from the surrounding protoplasm by the absence from all points of its wall, of granulation. Next a small protoplasmic button forms at some point of the wall and advances progressively into the vacuole, crowding its contents back against the sides, so that after a time it becomes a pyriform body surrounded by a clear layer (the vacuolic contents) and connected with the protoplasm by a pedicle. Little by little the pedicle becomes strangulated, the pyriform body thus finally becoming free. During this time it has acquired a membrane, and a filament is produced within it, evidently at the expense of its protoplasm, although Thélohan was unable to follow all the stages of the process. Around the capsule thus formed one finds the nucleus,<sup>3</sup> and débris of the protoplasmic globule which has given birth to the capsule. The nucleus remains most frequently attached to the capsule, but sometimes it becomes separated and is found engulfed in the sporoplasm. During development the capsules have no fixed direction, orientation taking place later.

*b. Development of the sporoplasm.*—The third mass becomes the sporoplasm. Very early 2 nuclei, generally near together, are seen. They persist to maturity. Thélohan was unable to determine whether these exist primitively in the sporoblasts (which would then contain 4 nuclei instead of 3, as Bütschli supposes) or whether they result from division.

*c. Development of the finished spore.*—The spores, until now rounded or oblong, very soon assume their definite and characteristic shape and acquire an envelope. The tail is folded against one side of the spore, becoming straight only after the rupture of the pansporoblast membrane, which latter persists a rather long time.

<sup>1</sup> Bütschli for *M. mülleri*; Balbiani for *M. ellipsoides* (see pp. 218, 223).

<sup>2</sup> Not rarely, especially in *Myxobolus ellipsoides*, 3 to 8 capsules are found. The constant association with each of a nucleus shows that their formation takes place in the usual manner. In this case the [pan]sporoblast without doubt incloses an abnormal number of nuclei. Sometimes it even seems probable that a single spore is formed instead of 2 (Thélohan). [It would be exceedingly interesting to ascertain whether in these cases the number of rejected nuclei is correspondingly less. Unfortunately, at present nothing is known on this point.]

<sup>3</sup> Thélohan here remarks that in a preceding work (Compt. Rend. Acad. Sci. Paris, 1889, cix, pp. 920-1, and Annal. de Microgr., 1890, II, p. 210) he considered these nuclei as belonging to the sporoplasm and attributed to them a different origin, an error which a study of the development has rectified.

## THE SPORE.

The myxosporidian spore always consists of at least 3 structures, viz: a shell, one or more capsules with filament, and the single mass of sporoplasm. In *Myxobolus* (p. 207) there is also sometimes present a fourth structure—the tail.

Pfeiffer<sup>1</sup> regards the myxosporidian spore as the equivalent of the individual falciform germs (*sporozoites*) of the *Coccidia*.

## THE SHELL.

This was noticed by even the earliest observers, who commented upon its most prominent features, viz: its extreme transparency and resistance to the strongest chemical reagents. Creplin<sup>2</sup> was the first to observe the separation of the valves after prolonged immersion in water. It is extremely probable that the shell substance is the same throughout the whole group, as we find the constant shell characters to be the micro-chemical ones, variation appearing to be rather structural than chemical. This substance is thin, very transparent, insoluble in the strongest acids and alkalies in the cold, certainly in some, and probably in most species destroyed by (soluble in?) concentrated sulphuric acid at its boiling temperature;<sup>3</sup> usually with little affinity for staining reagents. The shell possesses a minute pore (or pores) for the exit of the spiral filaments.

Two types of shell are (provisionally at least) to be distinguished. These are the bivalve shell, and a type in which no bivalve structure has been detected.

The first type comprises 2 subtypes, viz: (a) plane of junction of valves coincident with the longitudinal plane; characteristic of *Myxobolus*; and (b) plane of junction of the valves perpendicular to the longitudinal plane; characteristic of the *Cystodiscidae* and the *Chloromyxidae*.

The second type is found in the *Glugeidae* and in *Myxidium lieberkühni*.

*Tail*.—Confined within and described under the genus *Myxobolus* (p. 207).

## CAPSULES AND FILAMENTS.

## MORPHOLOGY.

*Capsule*.—Always pyriform, consisting of a thick, elastic, brilliant, ordinarily opaque wall encompassing a central cavity; wall drawn out

<sup>1</sup> Die Protozoen als Krankheitserreger, 1891, 2 ed., p. 8.

<sup>2</sup> Wiegmann's Archiv. f. Naturgesch., 1842, I, p. 63.

<sup>3</sup> Balbiani asserts (Journ. de Microgr., 1883, VII, p. 202) that boiling sulphuric acid does not affect the shell. This Bütschli (Ztschr. f. wiss. Zool., 1881, XXXV, p. 634) denies, stating that strong heating with sulphuric acid destroys entirely the shell substance. My own experience with several species tallies exactly with that of Bütschli.

anteriorly into a duct which pierces the shell near its anterior extremity, affording exit for the filament. Wall usually taking (sometimes retaining, sometimes yielding up upon washing out) stains, especially the nuclear. Thélohan<sup>1</sup> considers the substance composing the capsular wall identical with that forming the shell, as both stain in the same way with safranin. From this view I must dissent, as in my experience not only the optical character, but also all the prominent staining reactions, differ. In particular the capsules are *uniformly opaque*, the filaments never being visible through them, even in glycerin, while the shell is transparent in the highest possible degree. Further, in *Myxobolus macrurus* (other species were not tried) bismarck brown and fuchsin each stain the capsule without even tinting the shell.

Two reagents render the capsular wall transparent, thus permitting the filament to be seen coiled *in situ*. The first is iodine water (solution with potassium iodide). This reagent also causes extrusion of the filaments, sometimes even in alcoholic specimens (pp. 85, 120). The second is strong ammonia water. I have never seen it produce extrusion of the filament.

Bütschli<sup>2</sup> and Balbiani<sup>3</sup> have observed that when the filament is extruded there is ("as in the thread cells proper", Bütschli) a very marked diminution in the volume of the capsule, from which Bütschli infers that such extrusion is produced by the pressure of the stretched elastic capsular wall.

This may be the cause of filament-extrusion, but might it not equally well be interpreted as the result of such extrusion or, more properly, as a co-result with the latter of a general increase of intrasporal pressure? However this may be, it seems very probable that the filament-extrusion which takes place under the influence of such energetic dehydrants as sulphuric acid, glycerin, etc., is merely a physical effect, the result of the intense intrasporal endosmotic pressure. Thus in several species (among others, *Myxobolus transovalis*) sulphuric acid produces a pronounced swelling of the spore, extrusion (even in alcoholic specimens) of the filaments, and finally the expulsion of the capsules bodily, under an evidently great pressure. It can not, however, be denied that the action of iodine water is not thus explicable.

*Filament*.—Exceedingly tenuous, attached at its proximal extremity to the capsular wall, free at its distal extremity; usually coiled into a spiral; in this condition entirely inclosed within the capsule cavity. Capable of uncoiling and of extrusion (*via* the capsular duct) as a semi-uncoiled or a fully uncoiled (nearly or quite straight) thread whose length may be many times that of the spore. That the semiuncoiled condition is merely an intermediate stage between the fully coiled and the fully uncoiled condition, and is not a specific character, is shown

<sup>1</sup> Annal. de Microgr., 1890, II, p. 207.

<sup>2</sup> Ztschr. f. wiss. Zool., 1881, XXXV, p. 636.

<sup>3</sup> Journ. de Microgr., 1883, VII, p. 204.

by the occurrence of both in the same species under the influence of sulphuric acid. The other reagents which tend to produce filament-extrusion are caustic alkalis, hydrochloric and nitric acids, ether, glycerin, boiling water, mechanical pressure (e. g., the rolling of a mass of spores in an insufficiency of fluid, under the cover-glass), etc. As noted by Bütschli,<sup>1</sup> the extrusion in the latter case is apt to be more or less abnormal.

Concerning filament-extrusion in preserved material, Thélohan<sup>2</sup> says:

After the action of alcohol upon the spores the filament remains in the capsule and it becomes impossible to make it go out.

While not usual, extrusion does sometimes occur with alcoholic specimens, a certain (rather small) proportion of the spores emitting their filaments under the action both of sulphuric acid and of iodine water. In my experience the filaments appear usually not to have much affinity for stains; the capsule where stained, always shows a markedly lighter center. Kolesnikoff, however, found them to stain in *Myxobolus kolesnikovi*.

#### HOMOLOGY AND FUNCTION.

The capsules were first observed by Müller (see p. 241), who considered them the embryos.

In 1852 Leuckart<sup>3</sup> regarded these structures as fat globules. He says:

Also, they [the spores] contain some plain granules of a fatty quality, which are distinguished through their constant location in one or both poles.

In 1863 Balbiani<sup>4</sup> discovered the filament and its capability of extrusion. Regarding the spore as an adult cryptogam, he assigned to the filament the role of an antherozoid.

In 1875 Schneider<sup>5</sup> remarked that—

As regards a resemblance between the falciform corpuscles and the polar organs of the psorosperms of fishes, it is impossible for me to find it. \* \* \* The falciform corpuscles are not such sacks occupied by a slender filament rolled into a spiral.

Commenting upon Balbiani's views, Leuckart says:<sup>6</sup>

The signification of the elements is unknown, but it may be safely admitted that Balbiani's view, which sees therein an antherozoid, is without foundation. Perhaps it is to be regarded as an attachment apparatus.

He further remarks that a comparison of the capsules with the falciform corpuscles is excluded by Lieberkühn's and Balbiani's observations of the exit and anœroid movement of the sporoplasm.

<sup>1</sup> Ztschr. f. wiss. Zool., 1881, xxxv, p. 635; see *Myxobolus mülleri*, p. 219.

<sup>2</sup> Annal. de Microgr., 1890, II, p. 207.

<sup>3</sup> Archiv. f. physiol. Heilkde, XI, pp. 434-5.

<sup>4</sup> Compt. Rend. Acad. Sci. Paris, LVII, p. 159. This discovery has since been confirmed by numerous observers.

<sup>5</sup> Archiv. de Zool. Exper., Paris, IV, pp. 548-9. I have not seen a distinctly asserted comparison between the capsules and the falciform corpuscles to which this could refer, but such a comparison is implied by Leuckart's parallelism of *Myxidium* (?) sp. 102 (Archiv. f. physiol. Heilkde, 1852, XI, fig. 21 b) with the spore from the testicle of *Lumbricus*.

<sup>6</sup> Die Parasiten des Menschen, 1879, 2 ed., p. 247.

Upon the same subject Prof. Bütschli<sup>1</sup> remarks that:

Balbiani's view that they [the filaments] represent male fertilizing elements comparable to the antherozoids of the cryptogams, may be entirely rejected, as, apart from the general improbability of this view (which, moreover, is not further supported by actual observations), there are, at present known, no vegetable spermatozoon-like organisms whose structure permits of comparison with these nematocystoid polar corpuscles.

Prof. Bütschli<sup>2</sup> regards the capsule as comparable to the nematocysts of the Cœlenterates. This view is, he says, supported by its development, the filament being originally in the extruded condition and only subsequently becoming retracted and coiled.<sup>3</sup> Further Bütschli remarks that:

One might suspect that the capsular filaments serve for the attachment of the spores to other fishes or to the food of the same.

Taking the two together, I interpret Prof. Bütschli's meaning to be that morphologically they are nematocysts, but that here they function differently.

Replying to the preceding criticisms of his theory, Balbiani<sup>4</sup> says:

This last observer [Bütschli] compares with reason these filaments to the urticating organs or trichocysts of the Cœlenterates. But, knowing the signification of urticating organs, I admit that I do not well understand in what way these organs can serve psorospers which are completely immovable and do not nourish themselves, for one knows that the trichocysts have for their object only the paralysis of prey in order to render its capture more easy.

And further, among other repetitions of his theory, he says:

We have, in effect, here, all the phenomena of sexual union (*rapprochement*); first, the embrace (*rapprochement*) of two individuals; then the presence of a female element, the sarcodic globule, becoming free at that moment; and, finally, filaments which I have compared to antherozoids. In a word, the process recalls involuntarily to the observer a cryptogamic sexual generation. But these interpretations, although emitted with reserve, have drawn upon me on the part of Leuckart and Bütschli a severe criticism. These authors prefer to compare them to urticant organs. One can respond by asking them what would here be the physiological signification of urticant organs, which are offensive or defensive weapons. What would be, in these organisms, their rôle and utility? At all events the phenomena in question deserve to be studied anew. I was then as much, if not more, in the right to consider them as antherozoids, than Leuckart and Bütschli to make of them urticant organs. We had, I believe, equal reasons, the German observer and I, to sustain our interpretation.

Curiously enough Balbiani shows no indication of abandoning his antherozoid theory (on the contrary it is further elaborated by the designation of the sporoplasm as the "female element"), notwithstanding

<sup>1</sup> Ztschr. f. wiss. Zool., 1881, xxxv, p. 638; Bronn's Thier-Reich, 1882, I, p. 603.

<sup>2</sup> Bronn's Thier-Reich, 1882, I, pp. 599, 600.

<sup>3</sup> Bütschli's own observations for the *Myxosporidia*. The same very probable for *Hydra* (Jickeli, Morphol. Jahrb., VIII, p. 373). Without assigning any reason, Lutz doubts Bütschli's observation (Centralbl. f. Bakt. u. Parasitenkde, 1889, v, p. 87).

<sup>4</sup> Journ. de Microgr., 1883, VII, pp. 204, 277, 278.

the fact that at the same time he *practically abandons*<sup>1</sup> *his view of the adult nature of the "psorosperm."*

Kunstler and Pitres<sup>2</sup> think that the capsules "appear to be true nematocysts."

Ludwig<sup>3</sup> accepts the Leuckart-Bütschli attachment theory, regarding the filaments as probably organs of attachment. He says that though little is known as to the conditions under which filament-extrusion naturally occurs, spores kept long in water extrude their filaments, and adds:

Probably the filaments serve for the attachment of the spores, which have reached the water through the opened tumors of the fish, to any living or dead substances whatever.

Thélohan<sup>4</sup> comments upon Prof. Bütschli's view as follows:

Bütschli, after having severely criticised that idea [Balbiani's antherozoid theory], compares them to urticant organs. At the outset, as Balbiani observes, one can not see what could here be the rôle and the utility of urticating organs. Further, the filament of the polar capsules resembles but little those of the true nematocysts; after their exit they present most often a sinuous aspect, sometimes neatly spiral, which is far from recalling the appearance of the urticant filaments which shoot out abruptly from their capsules and present themselves under the form of rigid bayonets.

Mingazzini<sup>5</sup> takes a totally different view from other authors and one which it is impossible to reconcile with the present evidence. In the following passage, besides other errors, *the (capsular) filaments are confounded with certain shell-processes (ribbonettes)* described by Balbiani in *Myxobolus ellipsoïdes*, and further Bütschli's view (given above) of the function of the filament is curiously distorted:

Many observers have noted (in treating the myxosporidian spore with various reagents) the exit from the polar bodies of a very long filament, which normally is coiled within the polar body. As to the signification of this filament various opinions have been emitted. *Balbani thinks that it can serve as the organ of dispersal of the spore, functioning at the maturity of the latter in a similar manner to the elaters of the Elaterium spore.* Bütschli expresses the opinion that they can have the signification of urticant filaments.<sup>6</sup> But Balbiani has further observed that *in the mature spore these filaments are unwound and stand each around either the membrane of its own spore or around that of a neighboring spore*, and supposes that in the last case the filaments have the signification of copulating organs. Again, however, Bütschli, not entirely satisfied with his first interpretation, has thought that the function of urticant capsules for a spore which has a membrane resistant to acids and alkalies, is a kind of luxury, and that the filaments could serve to attach the spore to other fishes *or to feed it* [italics my own for errors].

From an analysis of the opinions it appears that none of them is entirely satisfactory, while, in my opinion, from what I have seen of the gregarinoid forms, it may be assumed that the polar bodies are nothing else than the embryos of the *Myxosporidia*, homologous with the falciform bodies of the gregarine and coccidian spores, on which view the filament of the polar body would be nothing else than the tail of the gregarinoid form which remains inclosed in the polar body while

<sup>1</sup> Journ. de Microgr., 1883, VII, pp. 198, 201, 276.

<sup>2</sup> Journ. de Microgr., 1884, VIII, p. 474.

<sup>3</sup> Jahresber. d. rhein. Fisch.-Vereins Bonn, 1888, p. 33.

<sup>4</sup> Annal. de Microgr., 1890, II, pp. 207-208.

<sup>5</sup> Boll. Soc. Nat. Napoli, 1890, IV, p. 163.

<sup>6</sup> See above (p. 86).

the mass of internal protoplasm would represent the residual nucleus (*nucleo di reliquat*) of the spore. The homology is demonstrated with all the greater probability, inasmuch as, as in the gregarine and coccidian spores, the number of the falciform bodies is constant with the species, so also in the *Myxosporidia* the number of the polar bodies is constant in the different species, and the residual nucleus would serve to feed them within the spore and perhaps to determine their exit at maturity. There would thus be explained what was seen by Balbiani, viz, the exit of the polar bodies at maturity without having recurrence to the forced interpretation of fecundation (which would not be constant) or to the unsatisfactory interpretations of Bütschli. We can thus see in the spore of the *Myxosporidia* all the parts that are encountered in that of the typical *Sporozoa* (the Gregarines and *Coccidia*), and in this way more easily discover the zoologic link which connects these groups with the *Myxosporidia*.

Perugia<sup>1</sup> accepts the Leuckart-Bütschli theory that the filaments are organs of fixation. He compares them to the long filaments of the eggs of parasitic Trematodes. This writer has, however, followed Mingazzini's error, and confounded the ribbonettes (described by Balbiani in *Myxobolus ellipsoides*, p. 223) with the capsular filaments.<sup>2</sup> It is necessary to direct special attention to this error or we shall soon find an elaborate table of structural synonymy a necessity. He says:

*Balbiani compares them to organs of dissemination such as the elaters of the Equiseti. Having afterward observed that sometimes this filament is coiled around another spore he saw in them an organ of copulation. Thélohan asserts that he has observed that many spores are destitute of such a filament and evinces an inclination to regard the filamentous organs as accidental productions(!)* [Italics my own for errors.]

Pfeiffer<sup>3</sup> regards the filaments as organs of movement or attachment, saying:

Probably this organ is no thread-cell, but serves for progression or attachment.

He<sup>4</sup> asserts that these structures also occur with the falciform germs of Miescher's tubes, and says that the spores of the *Myxosporidia* and *Sarcosporidia* are, according to his representation, not at all so widely different from one another. Further, in the description of fig. v, he says:

A well-developed falciform corpuscle; to the right the large colorable nucleus; to the left a noncolorable indefinite body with a beak-like process at the left pole (thread-cell?).

Thus, in spite of the unqualified statement in the text, there appears to be no certainty as to the nature of the structure in question. Turning to the figure, all that can be said is that it is entirely too indefinite to sustain the weight of the assertion of its capsular nature, against which view the verdict of "not proven" must be placed.

<sup>1</sup> Boll. Scientif., Pavia, 1890, xii, p. 137.

<sup>2</sup> Thélohan has recently pointed out Perugia's error (Bull. Soc. philomat. Paris, 1892, iv, p. 167).

<sup>3</sup> Die Protozoen als Krankheitserreger, 1 ed., 1890, p. 47; 2 ed., 1891, pp. 17, 132.

<sup>4</sup> *Ibid.*, 1 ed., pp. 47 (and footnote), 99, plate, fig. v; 2 ed., p. 133. It will be noted that Pfeiffer says nothing of, nor do his figures show, any extruded filaments. Nothing short of this could be accepted to prove the capsular nature of the body in question. See also pl. 7, fig. 5.



*Remarks.*—Balbiani, Thélohan, and Mingazzini appear to assume, as the basis for their criticism of Prof. Bütschli's view, that a structure morphologically a nematocyst must necessarily be urticant in function, in other words that the terms nematocyst and urticant organ are synonymous. This assumption is, to say the least, very dubious.

Concerning the homologies of the organs in question it is impossible to see how, as suggested by Mingazzini, they are to be brought into comparison with the falciform bodies of the gregarine and coccidian spores, inasmuch as (as noted by Schneider; see p. 85) the falciform bodies are not in any respects structurally similar to the myxosporidian capsules, and further it would seem (as implied in Leuckart's view above given) that the homology should lie between the protoplasmic structure in the one spore, and the protoplasmic structure in the other, whereas Mingazzini's parallel is between the protoplasm in the one and a structure which shows no evidence of such composition in the other, being apparently destitute of such characteristic protoplasmic structures as nuclei, vacuole, etc.

I can not, however, feel much greater confidence in their homology with the cœlenterate nematocyst. I can only interpret homology to mean such correspondence in development and structure as would (upon the evolution theory) imply descent from a common ancestor, and conversely no homology seems possible except in cases where (upon the same theory) one would be willing to admit such common origin.

In the present case, while the myxosporidian capsule shows a marked *histologic* resemblance to the cœlenterate nematocyst, it presents one very important difference, viz, that it appears and functions at an entirely different period of the life-history, i. e., it characterizes the spore and disappears before the adult stage is reached. Add to this the point cited by M. Thélohan (p. 87), and their (probable) utter uselessness to the myxosporidian spore as offensive or defensive weapons, and the parallel is by no means close enough to justify their assimilation to the nematocysts. The fact that the myxosporidian filament agrees (how closely?) with that of *Hydra* in having the filament first extruded and only subsequently retracted-coiled, does not seem sufficient to prove the morphological equivalence of the structures, as it might be possible that this mode of formation is the only one capable of producing the necessary elastic tension. Further,<sup>1</sup> "nematocysts" are known in some mollusks. All these facts render it very probable that these "nematocysts" have been independently evolved in the different groups. It may, however, well be a question to what extent of detail all of these "nematocysts" correspond.

As regards the function of the capsules and filaments, the only intelligible suggestion that has yet been made appears to be the view of Leuckart and Bütschli, which sees in them an apparatus for attachment. I can see no basis in the facts for Balbiani's antherozoid theory,

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<sup>1</sup> Lankester, E. Ray, 1878, *Encycl. Britan.*, 9 ed., VI, p. 108.

and no evidence in favor of Mingazzini's supposition that the capsules represent the embryos, the filaments functioning as flagellæ.<sup>1</sup>

On the contrary everything that we know about the *Myxosporidia* favors the view that the embryo is *not* the capsule but the sporoplasm, the presence in it of nuclei, of a vacuole, and of amœboid movements being quite conclusive. The most probable supposition in relation to the capsules is that they are accessory and temporary structures whose function is to secure attachment and perhaps a certain amount of motion, for the fulfillment of both of which objects they seem very well adapted. And it may be noted in passing that nematocystoid bodies are known which function for attachment, as well as those which function for stinging, etc.<sup>2</sup>

Before discussing the mode of action of the filaments, a few words may advantageously be devoted to the relative functions of the spore and myxosporidium stages.

(1) *Dispersal is absolutely necessary to the species*: This dispersal can take place only by the actual separation of myxosporidian individuals from one host and their migration to another, unless we adopt one of two very improbable suppositions, viz, either that they attach themselves to the eggs of the host and await their development or that they develop in an intermediate host which feeds upon the fish.<sup>3</sup>

(2) *The spore is the means by which such dispersal is effected*:<sup>4</sup> Thus Lieberkühn<sup>5</sup> saw some cysts "lost" and others opened, their contents escaping into the water. Also Ludwig and Railliet (p. 228) have observed the rupture of cysts *in situ* with escape of their contents. Thélohan<sup>6</sup> has seen the same occur with *Glugea anomala*; and in *Myxobolus ellipsoides* he saw cysts shell out entire and burst.<sup>7</sup>

<sup>1</sup> Mingazzini's description given above implies very strongly this idea as to the function of the filaments, nevertheless he does not distinctly so state. Compare here Lieberkühn's statement (Bull. Acad. Roy. Belg., 1854, XXI, pt. 2, p. 21) that the capsules, when extruded with the sporoplasm from the spore, show not the slightest trace of movement.

<sup>2</sup> In the body epithelium of the Ctenophora we find peculiar adhesive cells with uneven and sticky surfaces. Their bases are prolonged into spirally coiled contractile filaments.—(Arnold Lang's Text Book of Comparative Anatomy, London, 1891, pt. 1, p. 82.)

<sup>3</sup> The latter mode of change of host, though improbable, is not inconceivable. Still, everything seems to point toward the view that the whole life cycle from the attached spore in one generation to the liberated spore in the next, takes place in the same host.

<sup>4</sup> The only place where this view is distinctly stated is the following (Mlle. Leclercq, 1890, Bull. Soc. Belg. de Microsc., XVI, p. 101):

"On account of the presence of organs compared to nematocysts, but which seem rather elaters, one can believe that the spore is the disseminating form of the parasite, and that it can lead for some time a free life in the water." [Italics my own for errors.] Here we again see the unfortunate results of the dual signification of the term "filament."

<sup>5</sup> Müller's Archiv., 1854, p. 356.

<sup>6</sup> Compt. Rend. hebdom. Soc. Biol. Paris, 1892, IV, pp. 82-4.

<sup>7</sup> Annal. de Microgr., 1890, II, pp. 203-4. The observation was upon a spore habi-  
tant on the tench (*Myxobolus ellipsoides*?).

Finally that, in at least one species, dispersal could hardly take place by the myxosporidium is shown by Bütschli's observation<sup>1</sup> that in *Myxidium lieberkühni* that structure dies rapidly when removed from its natural habitat (the urine of the pike) to even "indifferent fluids."

(3) *The myxosporidium, on the other hand, is the post-embryonic, comparatively stationary, growth-reproduction stage:* There is little reason to suppose that there is ever any migration from one host to another during this stage. The evidence all points to the conclusion that after (and probably soon after) its attachment to the host, the valves of the spore separate, freeing the sporoplasm, which thenceforward is known as the myxosporidium. Thus Lieberkühn, Balbiani, Pfeiffer, and Perugia have all seen the sporoplasm leave the spore and exhibit amœboid movements.

Now, if this view as to their relative functions in the life-cycle be correct, the capsular filaments may conceivably serve in several ways. First, they may serve as a flagelliform swimming apparatus, a view that I think quite improbable, dispersal being more probably effected by currents, etc. Second, they may (and this is probably their most important function) serve for attachment.<sup>2</sup>

Further, if it be conceded that, after attachment, motion is necessary to the spore, the filaments might easily subserve such function either by a maximum extrusion, fixation of the tip, and a subsequent coiling-retraction (similar to that of the *Vorticella* stem), the spore in this case progressing "anterior" end foremost, or by a minimum extrusion followed by fixation of the tip and progressive uncoiling-protrusion, the spore in this case being pushed "posterior" end foremost. In *Glugea anomala*, which has but one filament,  $50\mu$  long, motion could hardly be effected in the latter way. But such motion is easily conceivable with the 2-capsuled (*Myxobolus*, etc.) spores; and if it were admissible to suppose that the final lodgment preliminary to reproduction is ever effected by the spore and not by the myxosporidium, the latter being liberated and growing *in situ* (a view which, however, the present evidence tends to negative), this backward motion would be the best possible for inserting the spore under a scale, especially for those species provided with a tail, which latter structure would form an efficient guide to such insertion. I incline, however, to the view that *the* function of the filament is attachment, and that the motion necessary for the attainment of a place for reproduction-encystment is effected by the liberated myxosporidium.

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<sup>1</sup> Ztschr. f. wiss. Zool., 1881, xxxv, p. 639.

<sup>2</sup> Perfectly consonant with this view is the observation of Bütschli (Ztschr. f. wiss. Zool., 1881, xxxv, p. 635) that the filaments are extruded in spores which are preserved a long time in water. For we thus see the floating spores ready for instant attachment to any object with which they may come into contact. A possible cause for such extrusion might perhaps be found in osmotic pressure (preponderant endosmosis from the surrounding water) from within. At any rate, it is difficult for me to attribute the rupture of the shelled-out cyst observed by M. Thélohan (see p. 221) to any other cause.

## SPOROPLASM

This was noted (but apparently regarded as a third capsule) by Müller,<sup>1</sup> and it appears in several of his figures. Subsequently Lieberkühn<sup>2</sup> observed its exit from the spore and its amœboid movements. He also notes its visibility within the spore.<sup>3</sup> These observations have been confirmed by Balbiani<sup>4</sup> and later by others (see pp. 263, 287).

The sporoplasm is extremely transparent, more or less granular, and contains nuclei (1 or more), sometimes a vacuole, and, at any rate in the genus *Myxobolus*, a variable number of brightly refringent granules.

*Nuclei*.—These were first demonstrated by Thélohan.<sup>5</sup> Their number is variable in the same spore, according to the stage of development. In *Myxobolus ellipsoides*, Thélohan demonstrated their origin by continuous division from a primitive single one. He further says<sup>6</sup> that all species studied by him (with the possible exception of the *Glugea* species, in which the small size of the spore prevented accurate determination) have shown 2 nuclei. This accords with my own observations.

*Granules* ("refringent globules," etc.).—These have been noticed in several *Myxobolus* species. They are described under that genus (see p. 209).

*Vacuole*.—This structure is of two types: (1) The noniodine-staining (*aniodinophile*) vacuole. This is known only in, and forms a marked characteristic of, the *Cryptocystes*. It is situated in the large extremity of the ovoid or pyriform spores and is unaffected by iodine. This structure was first observed, but not at that time recognized as a vacuole, by Thélohan.<sup>7</sup> Subsequently he recognized its true nature.<sup>8</sup> (2) The iodine-staining (*iodinophile*) vacuole. This is known only in, and forms a marked characteristic of, the *Myxobolidæ*. It is stained by iodine dark brown against a light yellow-brown ground. This reaction is best obtained with a dilute solution (aqueous, with potassium iodide). Further details are given under *Myxobolus* (p. 208).

<sup>1</sup> Müller's Archiv., 1841, p. 484, pl. 16, fig. 3 i, k; cf. fig. 5.

<sup>2</sup> Müller's Archiv., 1854, pp. 353-4, pl. 14, figs. 7, 8.

<sup>3</sup> Bull. Acad. Roy. Belg., 1854, XXI, pt. 2, p. 21.

<sup>4</sup> Compt. Rend. Acad. Sci. Paris, 1863, LVII, p. 160.

<sup>5</sup> Compt. Rend. Acad. Sci. Paris, 1889, CIX, pp. 920-21. For Perugia's confirmation, see *Myxobolus? merlucii* (p. 242). For Bütschli's "nucleus", see p. 219.

<sup>6</sup> Compt. Rend. Acad. Sci. Paris, 1892, CXV, p. 1092.

<sup>7</sup> Annal. de Microgr., 1890, II, p. 211, pl. 1, fig. 17a, b.

<sup>8</sup> Relative to the homology of the vacuole, Thélohan says:

"Is there any connection between the central vesicle and the rest of segmentation of the other *Sporozoa*? A certain fact is that the aspect of the plasmic mass of the spores of the *Myxosporidia* with that vesicle refractory to staining, and the nuclei disseminated in the protoplasm, recalls in a striking manner certain phases of development of the spores of the Gregarines."

## EXIT OF THE SPOROPLASM.

This, the last phenomenon of the spore stage, was first observed by Lieberkühn,<sup>1</sup> who described the process as seen in *Myxobolus sp.* 65. He also figured it as occurring in *M. sp.* 44. Gabriel<sup>2</sup> also describes (but in a somewhat different way, and possibly erroneously) the freeing of the sporoplasm in *Myxidium lieberkühni*. It was also observed by Balbiani<sup>3</sup> in *Myxobolus ellipsoides*, and recently it has been confirmed by Pfeiffer<sup>4</sup> and by Perugia.<sup>5</sup>

Bütschli,<sup>6</sup> however, entertains some doubt as to the supposed simplicity of the life-history based upon these observations. His objections are chiefly that this view leaves no function for the capsules to perform. As indicated above, this exit appears only to take place at a (for the capsules) *post-functional* period.

## III.—ZOOLOGICAL POSITION.

Gluge<sup>7</sup> regarded the spores of *Glugea anomala* as crystals modified by an unknown cause. He says:

It is known from the researches of M. Ehrenberg that the silvery color of fishes is produced by a great number of corpuscles of a crystalline structure and a form cylindrical and a little recurved. It appears to me extremely probable, from all that precedes, that the corpuscles contained in the cysts are only the crystals of the normal state, but changed by an unknown cause.

Müller<sup>8</sup> regarded the *Myxosporidia* as agreeing neither with the spermatozoa nor with the germs of developing animals, nor with the tailed *Entozoa* or *Cercariae*, and as deviating equally in structure from the known fungi parasitic upon animals; finally, through their form, structure, development, specific distinctions, and absence of motion, they deviate from all known normal and pathological cell formations. This observer<sup>9</sup> bestowed upon these anomalous forms the name of "psorosperms,"<sup>10</sup> recalling both the cutaneous "eruption" produced by them and the resemblance of the tailed spores to spermatozoa.

The credit of first suggesting a definite zoological position for the subclass is due to Creplin.<sup>11</sup> It will be seen that he was the originator of what may be called the "gregarine theory."

<sup>1</sup> Muller's Archiv., 1854, p. 354; Bull. Acad. Roy. Belg., 1854, XXI, pt. 2, p. 21.

<sup>2</sup> Jahres-Ber. schles. Ges. vaterl. Cultur f. d. J. 1879, LVII, p. 192.

<sup>3</sup> Compt. Rend. Acad. Sci. Paris, 1863, LVII, p. 160.

<sup>4</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., p. 47; 2 ed., 1891, p. 133.

<sup>5</sup> Boll. Scientif., Pavia, 1891, XIII, p. 23.

<sup>6</sup> Ztschr. f. wiss. Zool., 1881, XXXV, pp. 637-8; Bronn's Thier-Reich, 1882, I, p. 595.

<sup>7</sup> Bull. Acad. Roy. Belg., 1838, V, p. 776.

<sup>8</sup> Müller's Archiv., 1841, pp. 487, 488.

<sup>9</sup> Mlle Leclercq (Bull. Soc. Belg. de Microsc., 1890, XVI, p. 100) erroneously attributes the name to Gluge.

<sup>10</sup> Derivation furnished by Balbiani (Journ. de Microgr., 1883, VII, p. 145) as follows: ψωρα, mange; σπέρμα, seed.

<sup>11</sup> Wiegmann. Archiv. f. Naturgesch., 1842, I, pp. 65, 66.

Creplin says:

Nothing even remotely similar has ever been seen by me in the many kinds of small cysts which I have frequently found in the invertebrate animals and have examined for Helminths. Since, however, I have seen v. Siebold's fine Contributions to the Natural History of the Invertebrate Animals (Danzig, 1839) I believe I have found something analogous to them in the organisms discovered by v. Siebold in cysts in the small intestine of *Sciara nitidicollis*, which he terms *Navicellæ*. See ff. 63 and the accompanying figures on Tab. III. \* \* \* Although some features may appear to indicate a vegetable nature, the cyst bears distinctive marks of its animal nature. Cyst formation precedes spore formation, the spores perhaps originating from the granules seen in the cyst fluid, or perhaps by free formation within that fluid, or by production from the cyst-wall.

Dujardin<sup>1</sup> also suggested the correlation of the "psorosperms" with the Gregarines in the following:

"Perhaps it is necessary to range with these productions those that one frequently observes in the testicles of *Lumbrici*.

In 1851 Leydig<sup>2</sup> developed the gregarine theory at some length. In brief, his reasons were as follows:

On him they made the impression of gregarine-like bodies and he knew no weighty reason against this view. They consist of roundish vesicles or vermiform tubes with a delicate membrane, and semi-fluid contents with granule masses. Frequently they appear as if a special membrane had not yet been separated from the contents, in which case the gregarinoid bodies have in contour somewhat the appearance of segmentation spheres. The fact that they only show granules does not contraindicate their gregarine nature, nor does the absence of motion, as slight motions might have been present, and further in some Gregarines motion cannot always be detected. Further, all who have studied the Gregarines unite in regarding the spores (*Navicellenbehälter*) as proceeding from the Gregarine. But any one who has compared the pseudonavicellæ and the psorosperms will certainly admit the conclusion that the navicelle, Müller's psorosperm, and the forms discovered by him in the diseased air bladder of *Gadus callarias* form one series, the different members of which are related as the genera of a family.

Further Leydig, having, as he believed, demonstrated the Gregarines to be life-stages of *Filaria*-like nematodes,<sup>3</sup> says (pp. 232-233) that the *Myxosporidia* of the plagiostomes can perhaps also be brought into unison with these views, by similar connection with the round *Filaria*-like nematode which he found in the blood of several plagiostomes and in the parenchyma of various abdominal viscera (especially in the spleen-pulp) and rarely in the blood of the umbilical cord of embryos of *Mustelus laevis*.

Leuckart,<sup>4</sup> in 1852, accepting Leydig's view that the Gregarines were developmental stages of nematodes, regarded the "psorosperms" as forming similar developmental stages, this view being based upon

<sup>1</sup> Hist. Nat. des Helminthes, 1845, p. 645.

<sup>2</sup> Müller's Archiv., pp. 226-228.

<sup>3</sup> According to Mingazzini (Boll. Soc. Nat. Napoli, 1890, IV, p. 162, footnote 2) these filarioid forms are referable to *Trypanosoma*.

<sup>4</sup> Arch. f. physiol. Heilkde, XI, pp. 434-6.

the great similarity between the spores and the pseudonavicellæ. He says:

For the further fate of our psorosperms it is not without interest to observe that they frequently occur free in the bile passages, while on the contrary they are no longer to be found in the intestinal canal, in which they, however, incontestably arrive. May they not here develop directly into those round worms which we not rarely encounter in the intestinal canal of these fishes.

Charles Robin was the first to assert their vegetable nature. In his *Histoire Naturelle des Végétaux Parasites* (Paris, 1853, pp. 291-2, 321), he collected descriptions and figures of nearly all the previously described species, placing them (as a special tribe, the *Psorospermeæ*) under the Diatoms. He says:

Several facts have convinced me of the vegetable nature of these bodies. These are the entirely peculiar aspect of the species<sup>1</sup> that I have had under observation; the definite rupture of the coriaceous cells of which they are composed; the presence upon some of special opercles; their contents partly homogeneous, partly formed of drops of oil in suspension in a clear liquid; the solubility of the walls, which often occurs in concentrated sulphuric acid in the manner of cellulose (although they are not colored by iodine). Like Müller and Retzius \* \* \* I believe that these vegetables approximate by their form and general structure to the Diatoms, among other forms to *Navicula* and *Melosira*, etc., although they differ in the absence of silica in the walls. \* \* \* Like the *Diatoms* they can live either free or reunited into colonies. \* \* \* Although it is probable that the species described below will one day form at least two genera, \* \* \* I shall unite them provisionally [under one genus.]

Lieberkühn<sup>2</sup> in his first paper expressed the opinion that the "psorosperms" could not be, as Leydig supposed, Gregarines, inasmuch as they possessed no nucleus. In his second paper<sup>3</sup> he again rejects Leydig's view in so far as the innominate form (*Gen. incert. sp. 12*) found by him under the skin of *Gasterosteus aculeatus* is concerned, saying that:

This mode of origin [the process of spore formation] is so peculiar that we certainly can not reckon such formations among the Gregarines. Their size, absence of structure, occurrence in water, the importance for reproduction of the granules, and the observed young stages, all give rise to opinions but not to certain knowledge.

Further, it is doubtful, he says, whether any Gregarine lives in water, whereas in all probability the psorosperm animal does, and attaches itself to the skin merely for reproduction. That the "psorosperms" are not amœbæ is indicated by his failure, on careful investigation, to find any of them capable of taking up foreign bodies into their substance. Also, he was never able to find an amœba which had just attached itself to the skin preliminary to reproduction. He concludes by saying that his researches on the parasites of fresh-water sponges promise to throw light on this subject, as he has there found large psorospermiform bodies consisting of small and large globular

<sup>1</sup> *Psorospermia sciænæ-umbræ* Robin (see p. 166).

<sup>2</sup> Müller's Archiv., 1854, p. 5.

<sup>3</sup> *Ibid.*, pp. 357-367.

heaps, amœbiform corpuscles of the same size with precisely similar granules, which corpuscles protruded processes of various form, and finally much larger formations, containing, simultaneously, both fine granules and psorospermiform structures which, moreover, showed movements similar to those of the amœbæ.

*Myxidium lieberkühni* is, however, referred to the Gregarines. The presence of a membrane is not regarded as a character indispensable to the definition of a Gregarine, inasmuch as in the earthworm there exist forms possessing all the other characters of true Gregarines (viz, a similar nucleus, the same form and size of granules, the same albuminoid substance, and the same manner of movement), and also other forms showing a plain but proportionately smaller nucleus, no demonstrable membrane, and none or only extremely fine granules. These forms possess amœboid movements, without, however, having the ability to take up into their substance foreign bodies or coloring matters. These characters permit of their classification under no other group than the Gregarines. Whether they represent young stages of these or special species is immaterial. This much, however, is clear: the nondemonstration of a structureless membrane does not exclude them from the Gregarines. The same may be said of the failure of demonstration of a nucleus, as either it may exist in spite of such failure, or it may be destroyed by the manipulation preliminary to examination, or it may be present at some other period of the life-history. Further, the opinion has been several times expressed that nonnucleated Gregarines exist. May they not rather be amœbæ? From these organisms they are delimited by their inability to take up into their substance undissolved solid particles.

In 1863 Balbiani<sup>1</sup> expressed a decided opinion in favor of their cryptogamic nature and, regarding the spore as the adult organism, assigned to the filaments the function of antherozoids, a view which he supplemented in 1883 by the designation of the sporoplasm as a "female element."<sup>2</sup> He further considered the "elastic ribbons" of *Myxobolus ellipsoides* comparable to the elaters of the *Equisetum* spore and supposed that, in addition to effecting valve separation, they serve to maintain the contact of two individuals during what he considered a state of conjugation. These views he reaffirmed in 1866.<sup>3</sup>

In 1875 Schneider<sup>4</sup> placed himself on record in opposition to the current theory of the close relationship between the *Myxosporidia* and the Gregarines, saying that:

One knows that, under the name of *Psorosperms*, there have been united (rather by reason of taxonomic necessities than by the coördination of positive data and sufficiently precise elements) four things, (Gregarines, *Myxosporidia*, *Sarcosporidia*

<sup>1</sup> Compt. Rend. Acad. Sci. Paris, LVII, pp. 157-161.

<sup>2</sup> Journ. de Microgr., VII, p. 278.

<sup>3</sup> Journ. Anat. et Physiol., III, pp. 600-602.

<sup>4</sup> Archiv. de Zool. Expér., Paris, IV, pp. 548, 561, and Notes et Revue, pp. XL, XLI,



and *Coccidia*), which it is necessary, at least until further information is obtained, to regard as distinct.

He further says that he fails to see any homology between the myxosporidian capsule and the falciform bodies of the gregarine spore.

Giard (see p. 170) suggests that the relation of the "psorosperms" to the Gregarines may be parasitic and not genetic; *Lithocystis schneideri* is regarded as a vegetable.

In 1879 Leuckart<sup>1</sup> recorded his opinion against the gregarine nature of the *Myxosporidia*, remarking that:

It appears, however, scarcely permissible at present to unite these psorosperm-sacs with the Gregarines, not merely because they lack the shell-wall which surrounds the gregarine spore (*Pseudonavicellen-Behälter*) but still more because the formation of the psorosperms begins at a time when the organism is still more or less removed from its maximum size, and such formation progresses thence during the whole of the subsequent existence. What is divided with the Gregarines into two successive phases falls with the psorosperm-sacs into one.

In several papers<sup>2</sup> Gabriel refers the "psorosperms" to the Myxomycetes. In his myxosporidian paper<sup>3</sup> (upon *Myxidium lieberkühni*) he says that—

The *Myxosporidia* can not be Gregarines, as they lack (1) the definite typical form, (2) the differentiated membrane, (3) the nucleus, and (4) the monosporogenous centers. Further, they possess the following nongregarine characters: (5) the manifold peculiar protoplasmic movements, (6) the "thread-drawing" substance, (7) yellow pigment, (8) vacuoles, (9) polysporogenous centers. The importance of characters 1 to 4 demands the separation of the *Myxosporidia* from the gregarine phylum. Further, while Lieberkühn's opinion that a membrane is not essential to a Gregarine might be admitted, the essentiality of a nucleus is less easily waived, and the fact remains that no Gregarine is known which simultaneously lacks both of these structures. Little satisfactory when considered alone, characters 5 to 9 confirm the myxomycetoid affinities of the *Myxosporidia*, as they are analogous to many exclusively myxomycetoid characters. Moreover, in Lieberkühn's time many subsequently discovered myxosporidioid, myxomycetous, and mycetozoan characters were still unknown.

Too much stress should not be laid upon the absence of pigment in gregarine species, although it is not concealed that the presence of pigment (yellow, brownish yellow, dark brown, blackish brown) is highly characteristic of the Myxomycetes.

The *Myxosporidia* are, therefore, to be annexed (not subordinated) to the Myxomycetes. The fact that they do not display typical myxomycete characters must not, however, be ignored. Though nearly allied to the same phylum, they are phylogenetically of more recent date and represent a small, sharply defined group, intermediate between the Myxomycetes and the Gregarines, originating by progressive adaptation to restricted and new life conditions.

<sup>1</sup> Die Parasiten des Menschen, 2 ed., p. 245.

<sup>2</sup> Tagebl. d. 51 Versamml. d. deutsch. Naturf. u. Aerzte, 1878, pp. 51, 52; Tagebl. d. 53 Versamml. etc., 1880, pp. 82, 83; extracts, criticism, etc., Zool. Anzeiger, 1880, III, p. 572; Zoolog. Jahresber., 1880, I, p. 161; Journ. Roy. Mic. Soc. London, 1882, II, pp. 358, 359.

<sup>3</sup> Jahresber. schles. Ges. vaterl. Cultur f. d. J. 1879, LVII, pp. 188-195.

In 1881, as the result of an extended study of both *Myxosporidia* and Gregarines, Bütschli<sup>1</sup> expressed his opinion substantially as follows:

That the relation between the *Myxosporidia* and the Gregarines is no very intimate one is shown both by the structure of the myxosporidium and by that of the spore, and also by the mode of spore formation. In the last two respects the *Myxosporidia* can be compared with the Gregarines only in the most general way. There are, indeed, some observations (e. g., the dubious one of Claparède's on *Monocystis capitata* Leuck., and that of Gabriel on a Gregarine of *Julus*, the latter, however, too incomplete to serve as a basis for theoretic conclusions) which render a nonencysted (perhaps also an endogenous) spore formation in certain Gregarines not improbable. The possession in common of bivalve and tailed spore shells is an unimportant similarity. Above all, we have every right to regard the capsules as a character especially indicative of the *Myxosporidia*, and of these no gregarine spore has so far shown a trace, the two bodies found by Schneider in the *Adelela* spore being scarcely to be paralleled with them.

These conditions [the capsules] of the myxosporidian spore speak just as strongly against a close connection between the *Myxosporidia* and the Myxomycetes, as the spores of the latter possess no structures comparable to the myxosporidian capsule. The pigment found in a few *Myxosporidia* (*Myxidium lieberkühni*, etc.) is not to be compared to that of the Myxomycetes, as it is not of myxosporidian but of extraneous origin. Naturally, the Myxomycetes, especially in the simplest forms, show in their partly peculiar endogenous spore formation a certain similarity to the *Myxosporidia*, but such a similarity also exists between the Myxomycetes and certain *Rhizopoda*. Among the latter the *Myxosporidia* seem to possess some special relation with the interesting *Pelomyxa*, inasmuch as the latter possesses a great number of small nuclei, and in addition it is probable that it produces endogenously chlamydo-spores, which, however, show no trace of capsules. Further, in the determination of the systematic position of the *Myxosporidia* stress should be laid upon the capsules. From everything that we know they are comparable only to the thread cells, which latter are exclusively animal structures which recent investigations have shown to be present in the *Protozoa*. I do not conceal that this criterion, like the other barriers which have again and again been raised between the animal and vegetable kingdoms, may be erected only to be overturned through more penetrating research.

In 1890 Pfeiffer<sup>2</sup> unites into his family "*Sporidien*" the *Myxosporidia*, *Microsporidia*, and *Sarcosporidia*. He says:

As a transition to more dangerous parasites are next to be made known the *Sarcosporidia*, of which Miescher's tubes in the transversely striped muscles of the warm-blooded animals are already known to physicians, but which are also found exactly similar, only with differently shaped spores, e. g., in the flesh of the barbel.

Spore formation has, he says, no constancy, transitions being found towards more highly developed forms and also toward the lower members of the *Sporozoa*. Thus in the tench fully developed forms are found only upon the branchiæ and in the air-bladder. In the gall bladder and the cysts on the splenic artery, spore types are found which form, step by step, transitions to the simple pseudonavicellæ of the Gregarines and to the structureless ovoids of the microsporidian cysts of *Bombyx*, *Daphnia*, etc., and to the condition observed in coccidian

<sup>1</sup> Ztschr. f. wiss. Zool., xxxv, pp. 648-650; also Bronn's Thier-Reich, 1882, I, pp. 601-603.

<sup>2</sup> Die Protozoen als Krankheitserreger, 1 ed., pp. 25-27, 42, 48, 74.

infection of epithelium. The typical myxosporidian spore-form is accordingly not of such preëminent importance. Further:

Whether the differentiation of the *Sporidia*, heretofore principally based upon the structure of the spore, will permit itself to be maintained is a matter for zoologists. The following investigations show too often how little stress is to be laid upon this mark alone, and what variations occur through adaptation.

Compared with the Gregarines, the *Myxosporidia* show their lower position by the lack of constant body form.

In the second edition of the same work (1891, pp. 7, 8, 10) he reduces his family *Sporidia* to the rank of a subfamily of the family *Coccidia*. He regards the "psorosperm" as a resting spore, and says it may be the equivalent of the individual falciform germs of the *Sarcosporidia*. The capsules, he says, also occur in the sarcosporidian spore (see p. 88).

The following is, I think, a fair summary of the evidence:

The *Myxosporidia* differ from the remaining *Sporozoa* in the multi-nucleate amœbiform adult, the pausporoblastic spore formation, and especially in the capsulate spores, which never contain falciform germs. At the same time the consensus, and I believe the evidence, favors their retention in the *Sporozoa*, of which they form a rather aberrant subclass.

As regards the relation of the *Myxosporidia* to the Myxomycetes, is there any evidence that the myxosporidium is a plasmode? In the diagnosis of the myxomycete plasmode the following are the most important points:

(a) Actual observation of plasmode formation by fusion of individuals. Now, not only has this never been seen<sup>1</sup> in the *Myxosporidia*, but the multiple nuclei of the myxosporidium are known in several cases to (and in all probability always do) originate by the division of the primitive single one.

(b) The presence of various shades of red, brown, or black pigment. This has never been seen in the *Myxosporidia*. All pigment there found appears to be of extraneous origin.<sup>2</sup>

Add to this the differences in the methods of spore formation (and particularly the fact that spore formation in the *Myxosporidia* does not terminate the life cycle) and the further fact that, as Bütschli remarks, no known myxomycete spore has any structure comparable to the

<sup>1</sup> Of course it may hereafter be found, but it will be time enough to approximate the two groups when it is found.

Even if its existence were demonstrated (and, from sarcosporidian analogy, Pfeiffer regards it only as probable), the process described by Pfeiffer (Die Protozoen als Krankheitserreger, 1 ed., 1890, p. 34; 2 ed., 1891, p. 108; see also p. 227) in the muscles of the barbel could not possibly bear this construction, as the myxosporidium fusion here described is not zoologic, but secondary to common incapsulation, and is rather comparable to fusion of abscesses and ovarian cysts, where the adjacent walls disappear from pressure-atrophy, or otherwise.

This fusion process under pressure has also recently been observed by Korotneff (see p. 188).

<sup>2</sup> This statement must perhaps now be qualified; see pp. 77, 277.

myxosporidian capsule, and the evidence against the myxomycete theory becomes very strong.

#### IV. DISTRIBUTION.

From the practical standpoint there is no more important branch of the subject than the conditions under which the growth of the parasite takes place. Closely related to these conditions is its distribution as regards host and organ, space and season.

#### ZOOLOGICAL DISTRIBUTION.

The following table includes all the doubtful and true *Myxosporidia* (species 7 to 102) arranged zoologically by hosts. This arrangement reveals a few correlations between the taxonomic relations of the host and those of the parasite.

Host.	Scat.					Genus.								Stage known.		Species.	Species No.				
	Muscles.	External sur- face.	Solid organs.	Branchial cav- ity.	Air bladder.	Gill and uri- nary bladders, and urinary tubules.	Miscellaneous.	Genus incert.	Glugea.	Pleistophora.	Thelohanlia.	Myxobolus.	Chloromyxum.	Ceratomyxa.	Cystodiscus.			Sphaeromyxa.	Myxidium.	Cyst.	Myxosporidium.
Polychaeta: Alcyonella fungosa.....							periviscer- al cavity	X											X		X
Vermes: Nais proboscidea.....												X								X	X
Crustacea: Palemon rectirostris.....	interfibril- lar.										X									X	X
Palemon serratus.....	do.										X										
Palemonetes varians.....	X										X										
Crangon vulgaris.....	X										X										
Astacus fluviatilis.....	X										X										
Insecta: Lortia viridana.....							body cav- ity												X		X
Pisces: Squalus acanthias.....													X								
Galeorhinus galeus.....													X								
Galeus mustelus.....													X								
Do.....													X								
Scyllorhinus stellaris.....													X								
Scyllorhinus canicula.....													X								
Pristiurus melanostomus.....													X								
Squatina squatina.....													X								
Raja batis.....													X								
Do.....													X								
Raja clavata.....													X								
Torpedo torpedo.....													X								
Torpedo marmorata.....													X								
Dasyatis sp.....													X								
Dasyatis pastinica.....													X								
Cephalanthus aquila.....													X								
Leptocephalus conger.....													X								
Erimyzon succetta oblongus.....													X								
Do.....													X								
Cyprinus carpio.....													X								
Do.....													X								

\* "Myxosporidium" (name not in good standing; see p. 206).



<i>Do.</i>		"accidentally" in kidney.								×										×	<i>elegans</i> *	88
<i>Do.</i>	head-squamous.																			×	<i>sp. incert-transovalis</i>	53
<i>Notropis megalops</i>	subcutaneous.																			×		63
<i>Tinca tinca</i>																				×	<i>sp. incert</i>	13
<i>Do.</i>		spleen	branchiae	?	gall bladder.															×	<i>piriformis</i>	35
<i>Do.</i>		spleen, kidney, liver	?	do																×	<i>brachycystis</i>	37
<i>Do.</i>			branchiae	×	?	gall bladder.														×	<i>ellipsoides</i>	49
<i>Do.</i>	cornea		do																	×	<i>bicostatus</i>	48
<i>Do.</i>	scales																			×	<i>sp. incert</i>	38
<i>Chondrostoma nasus</i>	tongue roots.																			×	<i>do</i>	20
<i>Abramis brama</i>			branchiae																	×	<i>do</i>	7
<i>Do.</i>			do																	×	<i>do</i>	44
<i>Do.</i>			do																	×	<i>do</i>	45
<i>Alburnus alburnus</i>																				×	<i>obesus</i>	57
<i>Do.</i>			branchiae																	×	<i>oviformis</i>	42
<i>Misgurnus fossilis</i>		kidney																		×	<i>piriformis</i>	35
<i>Rhandaia sebæ</i>			lining membrane.																	×	<i>linearis</i>	78
<i>Pimelodus clarias</i>																				×	<i>inequalis</i>	36
<i>Synodontis schali</i>	head																			×	<i>strongylurus</i>	73
<i>Pseudoplatystoma</i>			branchiae																	×	<i>linearis</i>	78
<i>Do.</i>			branchiae																	×	<i>sp. incert</i>	47
<i>Ancistrus melas</i>	dorsal fin																			×	<i>cf. linearis</i>	77
<i>Oreoginus fora</i>																				×	<i>zschokkei</i>	68
<i>Do.</i>	interramuscular tissue.																			×		81
<i>Do.</i>	interstitial muscular connective tissue.																			×	<i>kolesnikovi</i>	
<i>Do.</i>	×																			×		
<i>Do.</i>			branchial arches.																	×	<i>sp. incert</i>	82
<i>Do.</i>			do																	×	<i>do</i>	76
<i>Lates lucius</i>																				×	<i>spheralis</i>	60
<i>Do.</i>			do																	×	<i>sp. incert</i>	41
<i>Do.</i>			do																	×	<i>cf. creplini</i>	69
<i>Do.</i>	ovary																			×	<i>schizurus</i>	79
<i>Do.</i>	orbit																			×		

\* *Sphaerospora*.







## ORGANAL DISTRIBUTION.

## ORGANAL DISTRIBUTION OF THE GENERA AND SPECIES.

Perugia<sup>1</sup> remarks that there is a marked difference in seat between the *Myxosporidia* of marine and those of fresh-water fishes. In marine fishes they occur principally in the gall bladder, while in fresh-water fishes their organal range is much wider. The finding of cysts on the branchiæ of the marine genus *Mugil* (see p. 213) rather corroborates than contradicts this view, inasmuch as these fishes ascend rivers for a long distance, and those which yielded the myxosporidian cysts also yielded a Trematode of a genus peculiar to fresh-water fishes, viz, *Tetraonchus vanbenedenii* Par. & Per.

The organal distribution of the *Myxosporidia* is very extended. The following points are of special interest, and comprise the principal anomalies of distribution not covered by the tables below.

*Nervous system*.—No species have ever been reported.

*Testicle*.—No species have ever been reported, a fact which,<sup>2</sup> considering their frequency in the ovary, is very surprising (cf. the presence of "*Myxosporidium*" bryozoides on the spermatoblasts of *Aleyonella fungosa*; see p. 187).

*Superficial tract*.—General similarity of conditions, histologic structure, and fauna justify the fusion of the general surface, skin, scales, the branchiæ, the eye, and the air bladder into one tract. The characteristics of this tract are principally the predominance of connective tissue, and (?) a relatively larger supply of oxygen (see p. 224).

*Air bladder*: Only two species are known from this seat. Both of these occur in *Cyprinidæ*, in which the bladder communicates freely with the intestine, and hence presumably contains oxygen. This fact, the histologic similarity, and the fauna suggest very strongly the propriety of including the air bladder in the external tract. The species are *Gen. incert. sp. 15* and *Myxobolus ellipsoides*.

*Intestinal canal*.—They would appear to be very rare here. I am not aware that any species has ever been reported from the lumen, the nearest approach to it being one (*Myxidium?* sp. 102) from the bile-ducts. And yet such a species as the last must almost certainly find its way into the intestine; probably, however, as separated, single spores, very difficult to find. In addition, *Myxobolus ellipsoides* and *M. sp. 51* (the latter from the wall), and finally *Gen. incert. sp. 17* (which, however, may or may not be myxosporidian) occur on, or in the intestine.<sup>3</sup>

<sup>1</sup> Boll. Scientif., Pavia, 1890, XII, p. 139.

<sup>2</sup> As remarked by Thélohan (Annal. de Microgr., 1890, II, p. 197).

<sup>3</sup> The fact that *M. ellipsoides* and *M. sp. 51* are, of all the *Myxosporidia*, the species having the widest organal distribution, should not be lost sight of in considering their presence in unusual seats.

*Liver* (exclusive of gall bladder and ducts). But two species are known here, and these are the two which have the widest organal range, viz: *Myxobolus ellipsoides* and *Myxobolus* sp. 51.

*Kidney*.—In only a few instances has any distinction been made between the stroma of the kidney and the tubules. It seems, however, not improbable that, as regards organal distribution, a distinction should be made, and the tubules be regarded as a part of the hollow fluid-filled urinary tract, the stroma forming a solid connective tissue seat. The following occur here:

"Kidney": *M. piriformis*, *M. brachycystis*, *M. mülleri*, *Myxobolus* sp. 51, *M.* ? sp. 65, *M. diplurus*.

Renal tubules: *Myxobolus brevis*, *M. medius*, *Chloromyxum* (*S.*) *elegans*, *C. (S.) ohlmacheri*.

*Spleen*.—This organ has furnished: *Myxobolus piriformis*, *M. brachycystis*, *M. Ellipsoides*, *M.* sp. 51.

*Ovary*.—From this are known: *Myxobolus mülleri*, *M.* sp. 51, *M. brevis* (2 hosts), *M. medius* (2 hosts), *M. cf. creplini*, *Chloromyxum* (*S.*) *elegans* (2 hosts), *C.* sp. 91.

*Excretory tract*.—For purposes of organal distribution, the gall and urinary bladders should be considered together, as they present practically identical environmental conditions, both being internal (which means a uniform temperature) and both being fluid-filled. To these cavities may perhaps be added, as exhibiting similar conditions, the bile-ducts and the renal tubules.

If, now, we consider this tract as a whole, we find that its rich and peculiar fauna stands in strong contrast to the species inhabiting the remaining organs. For we find absolutely confined to it the following: The *Chloromyxidæ* except only *Chloromyxum dujardini*, the *Cystodiscidæ*, except the insecticolous *Cystodiscus* ?? *diploxyis*, and the *Myxididæ*. Besides these, only the following species occur in this tract:

(a) In the gall bladder: *Genus incert. sp. 9*, "*Myxosporidium*" *congrisi*,<sup>1</sup> *Myxobolus* ? *merlucii*.<sup>2</sup>

(b) In the renal tubules: *Myxobolus brevis*, *Myxobolus medius*.

In the following table all the species—47 in number—whose generic references are fairly certain and whose seats are known, are compared as regards their organal distribution. The unit adopted is the occurrence of 1 myxosporidian species in 1 organ of 1 host. The number of such "occurrences" is shown for each species by the Roman, and for each genus by the Arabic numerals.

<sup>1</sup> Spore unknown (genus? See pp. 110, 182).

<sup>2</sup> Generic reference, in the almost entire absence of a description, by no means certain.

## Organel distribution.

Muscles.	Superficial tract.				Intestine.	Liver (except gall bladder and ducts).	Spleen.	Ovary.	Kidney (except renal tubules).	Excretory tract.				Genera and species.	Species No.
	Body surface, skin, scales, fins, subcutaneous tissue, cornea, eye.	Branchial cavity (lining membrane of), branchial lamellae, pseudo branchiae.	Air bladder.							Renal tubules.	Gall bladder.	Urinary bladder.	Bile ducts.		
I		3												Glugea:	
I		III												destruens.....	27
I														anomala.....	28
I														Pleistophora:	
I														typicalis.....	29
5														Thelohania:	
II														contejeani.....	30
I														octospora.....	31
I														giardi.....	32
I														macrocystis.....	33
3		10	14	1	1	2	2	7	4*	4				Myxobolus:	
I														kolesnikovii.....	81
I														sp. incert.....	82
I														sp. incert.....	51
														oblongus.....	54
														lintoni.....	55
														transvalis.....	63
														strongylurus.....	73
														monurus.....	74
														macrurus.....	75
														cf. linearis.....	77
														schizurus.....	79
														oviformis.....	42
														mülleri.....	46
														sp. incert.....	76
														globosus.....	62
														sp. incert.....	45
														linearis.....	78
														psorospermicus.....	80
														ellipsoides.....	49
														piriformis.....	35
														cf. creplini.....	69
														brevis.....	70
														medius.....	71
														diplurus.....	83
3		10	14	1	1	2	2	7	4*	4				Total "occurrences"	
														of vacuolate species.	
			2					2	1*	3	13	1		Chloromyxum:	
			II					II	I*	II				(S.) dujardini.....	92
										I				(S.) elegans.....	88
										I				(S.) ohlmacheri.....	89
														incisum.....	93
														leydigii.....	94
														fluviale.....	95
														mucronatum.....	96
														Ceratomyxa:	
														arcuata.....	84
														agilis.....	85
														appendiculata.....	86
														sphaerulosa.....	87
														Cystodiscus:	
														immersus.....	97
														Sphaeromyxa:	
														balbianii.....	99
														Myxidium:	
														incurvatum.....	101
														lieberkühni.....	100
														sp. incert.....	102
			2					2	1*	3	27	2	1	Total "occurrences" of nonvacuolate species.	

\* "Kidney." As no distinction has been made between the kidney stroma and the tubules, these 4 cases are, as regards the present discussion, indeterminate.

† As regards the present question, it matters not whether eventually this species proves to be a *Myxidium* or to belong to some other of the genera with capsules in two separated groups, as all of these genera are nonvacuolate.

These data may be summarized as follows:<sup>1</sup>

Species.*	Species of <i>Phaenocystes</i> compared.	
	Non-vacuolate.	Vacuolate.
Confined to excretory tract.....	14	0
Common to both tracts .....	1	2
Limited to nonexcretory tracts.....	1	22

"Occurrences."	Number of "occurrences."	
	Non-vacuolate species.	Vacuolate.
Total*.....	37	44
In excretory tract .....	33	4
In nonexcretory tract. ....	4	40

\* Omitting the dubious "kidney" species and occurrences, and the somewhat questionable occurrence of *Myxobolus ellipsoides* in the gall bladder.

#### ORGANAL DISTRIBUTION OF THE VACUOLE.

From an examination of the above table it will be seen that the range of the genus *Myxobolus* throughout the organs is a wide one, *but that it is almost strictly complementary to that of the Chloromyxidæ, Cystodiscidæ, and Myxidiidæ.*

The real significance of these peculiarities of organal distribution lies, however, not so much in the peculiarities of generic-organal distribution, interesting as these are, as in the fact that *these limits of the distribution of the genera in the organs almost exactly coincide with the limits of the presence of the iodophile vacuole in the subclass*, nearly all of the nonvacuolate *Phaenocystes* being confined to the excretory tract, while nearly all the vacuolate *Phaenocystes* are absent from this tract.

Two questions immediately suggest themselves:

1. Is it possible that the function of the vacuole is here even remotely shadowed? The constancy of the vacuole in the spore and the inconstancy of vacuoles ( ? genetically related ) in the myxosporidium would seem to indicate that it functions during the spore stage. One supposition which suggests itself is that in some way it might subserve oxygenation, but it is more probable that it serves as a food reservoir for the sporoplasm (cf. Thelohan's comparison of its micro-chemical reactions with those of glycogen; p. 208 ). Unfortunately the origin of the structure and the phenomena of its disappearance after the exit of the sporoplasm have not been worked out.

<sup>1</sup> If the dubious occurrence of *Myxobolus ellipsoides* in the gall bladder be excluded as not proven. In any case the exceptionally wide organal range of this species should be considered in estimating the value of its occurrence in unusual seats.

2. Are the present generic references of some species correct and are their structural characters accurately determined? While at present the force of analogy is not so absolutely overwhelming as to justify a positive assertion, I strongly suspect that species of genera now indeterminate will ultimately tend to range themselves in accordance with the lines indicated: i. e., that species inhabiting gall bladders (Perugia's "*Myxosporidium*" *congri*, for example) will be found to be referable to nonvacuolate genera.

#### GEOGRAPHICAL AND SEASONAL DISTRIBUTION.

Out of 76 species of hosts and 96 forms of *Myxosporidia* (true and doubtful; species 7 to 102) localities are known for only 27 species of hosts and 19 forms of *Myxosporidia*, and many of the localities are so vague that they amount to little. In the hope that future descriptions will supplement this glaring deficiency, a table is given showing all the localities and dates of collection heretofore reported.

The condition of the data as regards season is even worse than that referring to locality. Even an approximate date of collection is known in only about 25 per cent of the forms, and yet of all classes of data this is certainly one of the most important. Many of the statements are general in the extreme (e. g., "summer"), and in not a single instance has the temperature of the water been recorded.

#### Geographical and seasonal distribution.

Locality.	Date.	Host.	Species.	Species No.
Asia:				
Irtisch .....	May, June .....	<i>Perca fluviatilis</i> .....	<i>Myxobolus</i> sp. incert .....	66
Europe:				
Russia—				
Don .....	First of winter, May, June.	<i>Stizostedion lucioperca</i> ...	<i>Myxobolus</i> sp. incert .....	61
Germany—				
Near Kiel*		<i>Gasterosteus aculeatus</i> ...	Gen. incert. sp. ....	22
Exact locality?		<i>Carassius carassius</i> .....	<i>Myxobolus</i> sp. incert .....	56
Weser .....		<i>Lucius lucius</i> .....	<i>Myxidium lieberkühnii</i> ..	100
Elbe .....		do .....	do .....	100
Rhine .....		do .....	do .....	100
Do .....		<i>Barbus barbus</i> .....	<i>Myxobolus</i> sp. incert .....	51
Saar .....		do .....	do .....	51
Do .....		<i>Lucius lucius</i> .....	<i>Myxidium lieberkühnii</i> ..	100
Mosel .....		<i>Barbus barbus</i> .....	<i>Myxobolus</i> sp. incert .....	51
German rivers .....	May, June .....	<i>Stizostedion lucioperca</i> ...	<i>Myxobolus</i> sp. incert .....	61
Do .....	do .....	<i>Leuciscus rutilus</i> .....	<i>Chloromyxum dujardini</i> ...	92
Do .....	May, June; May 8, 1875; Jan. 31, 1839.	do .....	<i>Myxobolus cycloides</i> .....	58
Do .....	May, June .....	<i>Perca fluviatilis</i> .....	<i>Myxobolus</i> sp. incert .....	66
France:				
Roscoff .....	Mar. 15 to Nov. 15 ad max.: July 15 to Aug. 31.	<i>Palæmon serratus</i> .....	<i>Thelohanias octospora</i> .....	31
Do .....		<i>Onus tricaratus</i> .....	<i>Sphæromyxa balbianii</i> ...	99
Do .....	August, 1892.	do .....	<i>Ceratomyxa arcuata</i> .....	84
		do .....	<i>Myxidium incurvatum</i> ...	101
		<i>Onus maculatus</i> .....	<i>Sphæromyxa balbianii</i> ...	99
		<i>Crenilabrus melops</i> .....	<i>Myxobolus mülleri</i> .....	46
		<i>Blennius pholis</i> .....	<i>Myxidium incurvatum</i> ...	101

\* The mention of this locality affords the only chance of an inferential correlation of this form with some one of the others known to live on the same fish.

## Geographical and seasonal distribution—Continued.

Locality.	Date.	Host.	Species.	Species No.
Europe—Continued.				
France—Continued.				
Roscoff .....	Aug. and Sept., 1892.	Lophius piscatorius .....	Ceratomyxa appendiculata.	86
Concarneau .....	Mar. 15 to Nov. 15; ad max. July 14 to Aug. 31.	Syngnathus æquoreus .....	Myxidium incurvatum ..	101
	Sept., 1892.	Palæmon serratus .....	Thelohania octospora ..	31
Le Croisic .....	Mar. 15 to Nov. 15; ad max. July 15 to Aug. 31.	Dasyatis pastinica .....	Ceratomyxa agilis .....	85
	Aug. and Sept., 1892.	Blennius pholis .....	Myxidium incurvatum ..	101
		Palæmon rectirostris .....	Thelohania octospora ..	31
Seule River .....		Lophius piscatorius .....	Ceratomyxa appendiculata.	86
Marne River .....		Phoxinus phoxinus .....	Myxobolus sp. incert. ....	53
Department of Doubs.		Barbus barbus .....	do .....	51
Valéry-au-Caux .....	Aug., 1891 .....	Astacus fluviatilis .....	Thelohania contejeani ..	30
Vilaine, at Rennes.	Aug., 1891 .....	Galeorhinus galeus .....	Ceratomyxa sphaerulosa ..	87
		Galeus mustelus .....	do .....	87
		Leuciscus erythrophthalmus.	Chloromyxum dujardini ..	92
Boulogne .....		Crangon vulgaris .....	Thelohania giardi .....	32
Italy:				
Mincio, near Verona		Palæmonetes varians .....	Thelohania macrocystis ..	33
Mediterranean Sea				
(? near Cagliari,		Scylliorhinus canicula .....	Chloromyxum leydigii .....	94
Island of Sardinia;		Squalus acanthias .....	do .....	94
See Müller's Archiv., 1851, p. 223).		Squatina squatina .....	do .....	94
		Torpedo torpedo .....	do .....	94
	Aug., 1890 .....	Leptocephalus conger .....	Gen. incert. ("Myxosporidium") congri.	11
	April, May .....	Coregonus fera .....	Myxobolus zschokkei .....	68
Europe (unknown localities).	Beginning of February, 1892	Lucius lucius .....	Myxobolus cf. creplini .....	69
	May, June .....	do .....	Myxobolus schizurus .....	79
	March 14, 1837 .....	Acerina cernua .....	Myxobolus creplini .....	72
	Aug. 13, 1890 .....	Merlucius merlucius .....	Myxobolus merlucii .....	64
Africa:				
Nile .....		Labeo niloticus .....	Myxobolus unicusculatus	34
		Synodontis schal .....	Myxobolus strongylurus ..	73
North America:				
Massachusetts: Atlantic, at Woods Holl.	Aug. 20, 1889; Aug. 1, 1892.	Cyprinodon variegatus .....	Myxobolus lintoni .....	55
New Jersey: Near Woodbury.		Aphredoderus sayanus .....	Myxobolus monurus .....	74
Virginia: Four-mile Run (tributary Potomac River), near Carlin's.	June 29, 1892 .....	Phoxinus funduloides .....	Myxobolus transvalis .....	63
North Carolina: Kingston.		Erimyzon sucetta oblongus.	Myxobolus oblongus .....	54
Do .....		do .....	Myxobolus globosus .....	62
South Carolina: Columbia.	March 21, 1880 .....	do .....	do .....	62
Mississippi: Tributaries Fox River.		do .....	do .....	62
Do .....		do .....	Myxobolus oblongus .....	54
Texas: Neches River, 14 miles east of Palestine.	Nov. 24, 1891 .....	Hybognathus nuchalis .....	Myxobolus macrurus .....	75
Iowa: Storm Lake .....	Aug. 23, 1890 .....	Ameiurus melas .....	Myxobolus cf. linearis .....	77
Illinois: Sycamore, De Kalb County.	Sept., 1892; July, 1893.	Bufo lentiginosus .....	Chloromyxum ohlmacheri ..	89
Ohio: Black River, Lorain County, 6 m. above Lake Erie.	Sept. 1, 1890; Oct. 5, 1891.	Notropis megalops .....	Genus incert. sp .....	13
South America:				
Guiana .....		Pimelodus clarias .....	Myxobolus inequalis .....	36
Surinam .....		do .....	do .....	36
South American rivers.		Rhamdia sebæ .....	Myxobolus linearis .....	78
Do .....		Pseudoplatystoma fasciatum.	do .....	78
Brazil (1 locality) .....		Bufo agui .....	Cystodiscus immersus .....	97
(2 localities) .....		Cystignathus ocellatus .....	do .....	97

V.—CLASSIFICATION OF THE MYXOSPORIDIA.<sup>1</sup>

Although several times previously authors had proposed generic names (apparently merely because the forms looked quite different, and, if we may judge from the absence of even a single generic definition to support any of the generic names, probably without any clear idea of the direction of generic lines) the first serious attempt at classification of the subclass was made by Thélohan.<sup>2</sup> The following is Thélohan's primary classification:

*Myxosporidians.*

Spores ..	{	Form variable....	{	Pyriform; capsule 1, at pointed extremity; vacuole 1, aniodinophile, at large extremity.	I. <i>Glugeidians</i> .		
				{	No vacuole; capsules 2 or 4.	Capsules 2.. II. <i>Myxidians</i> .	
					{		Capsules 4.. III. <i>Chloromyxans</i> .
						Vacuole 1, iodinophile. Capsules 1-2.	IV. <i>Myxobolans</i> .

The 3 principles laid down by him as a basis for classification may be thus summarized:

1. The habitat furnishes no sound basis for specific distinctions.

Here the following judicious criticism by Thélohan may be quoted:

Beyond the difference of their habitat, Perugia mentions no other characters which enable him to distinguish specifically the organisms that he has observed. But the habitat can not serve as a criterion, for, in addition to its being a fact entirely removed from the morphologic, histologic, and developmental characters of the parasite, it frequently happens that the same form lives at the expense of very different hosts, and, besides, a myxosporidian habitually parasitic on one particular host can accidentally invade a different species.

The conditions under which the parasite is encountered can not better be taken as a distinctive character, for the same species can present itself under very different states; for example, under the form of small, well-circumscribed tumors, or an irregular infiltration of the tissues.

There is little to add to this, except the hope that it may succeed in directing future investigations toward the parasite rather than the host.

2. The myxosporidium affords no taxonomic criteria.

The myxosporidium exhibits characters that are too nearly identical and too little contrasted to serve as bases for specific determinations. It is, however, possible and advantageous to take account of it, especially in the forms living free in the internal cavities, in which forms its differentiations are much more marked.

3. The spores alone (at least in the present state of our knowledge) offer characters suitable to serve as a basis for classification.

By noting the differences of form and size of these elements, the number of their

<sup>1</sup>The classification given below has already been published as a preliminary note in the Bulletin of the Commission for 1891 (XI, pp. 408-412). The present discussion contains everything there given with some amplifications.

<sup>2</sup> Bull. Soc. philomat. Paris, 1892, iv, pp. 165-178.



polar capsules, by taking account of the presence or absence of a vacuole in the plasma, of their number in the [pan]sporoblasts, one can, I believe, succeed in obtaining elements sufficient for an attempt of this kind.

And further:

I do not pretend to give a final classification of these organisms; I have wished only to furnish a means, a provisional means, for assigning to the species that may be discovered, a place in accord with their affinities; and above all I have wished, if not to terminate, at least to diminish the confusion which results from the arbitrary and vague manner in which all species have been designated; a confusion which I have only too often had occasion to recognize since I have studied these parasites, and which I believe adds a serious obstacle to the progress of our knowledge in their direction.

Upon the above extracts no criticism is needed. As far as they go they express exactly the conclusions at which I had independently arrived.

In any case, there can be no question as to the propriety of drawing a trenchant line between the "Glugeidians" of Thélohan, and the remaining *Myxosporidia*. This primary division (foreshadowed as early as 1890 by Thélohan)<sup>1</sup> can not, however, rest upon so comparatively unimportant a character as the outline of the spore. I have regarded it as of ordinal value, defining the two orders thus:

I. *Cryptocystes*. *Myxosporidia* in which the pansporoblast produces many (at the fewest 8) spores; the last minute, without distinct symmetry, with a single capsule; type (and only) family, *Glugeidæ*.

Etymology: *κρυπτός*, concealed; *κύστις*, capsule.

II. *Phanocystes*. *Myxosporidia* in which the pansporoblast produces few (at the most 2) spores;<sup>2</sup> the last relatively large, with distinct symmetry and 2 or more capsules;<sup>3</sup> type family, *Myxobolida*.

Etymology: *φαίνω*, I appear; *κύστις*, capsule.

Thélohan subdivides the *Phanocystes*<sup>4</sup> thus:

No vacuole: 2 or 4 capsules.....	$\left\{ \begin{array}{l} 2 \text{ capsules. II. Myxidians.} \\ 4 \text{ capsules. III. Chloromyxans.} \end{array} \right.$
1 iodophilous vacuole; 1 or 2 capsules.....	$\left. \right\} \text{..... IV. Myxobolans.}$

While the structure of the sporoplasm is of the utmost importance and the presence or absence, and the micro-chemical reactions of the vacuole are undoubtedly its most important taxonomic features, to obtain

\* <sup>1</sup> He says (Annal. de Microgr. II, p. 205):

"It is necessary to distinguish in the *Myxosporidia* two types of spores; the one of small size, always ovoid, and deprived of polar capsules; these Gluge discovered in the stickleback. The others, with which the authors have principally occupied themselves, are distinguished by their more considerable size, the different forms which they present, and by the presence of capsules."

<sup>2</sup> Three asserted in one species by Leydig (Müller's Archiv., 1851, p. 229).

<sup>3</sup> Except *Myxobolus unicusculatus* and *M. piriformis*. This qualification is omitted by Braun (Centralbl. f. Bakt. u. Parasitenkde, 1884, XVI, p. 86).

<sup>4</sup> For the classification of the *Cryptocystes*, see p. 190.

a satisfactory classification of the order it will be necessary to utilize additional characters, in particular those connected with spore topography and spore symmetry. This brings us to a consideration of the

#### SYMMETRY OF THE MYXOSPORIDIAN SPORE.

Considering the importance of the presence or absence of symmetry throughout the animal kingdom, it is strange that no attention has heretofore been paid to this feature of the myxosporidian spore. These bodies exhibit four varieties of symmetry, viz:

1. *Absence or obscurity of symmetry*.—This is found in the *Cryptocystes*. Antero-posterior symmetry is certainly absent; bilateral and supero-inferior symmetry (or asymmetry) obscure.

2. *Bilateral symmetry* (symmetry around the vertical plane). Present in all genera of *Phænocystes* except *Ceratomyxa*,<sup>1</sup> which is asymmetric as regards the position of the sporoplasm.

3. *Supero-inferior symmetry* (dorso-ventral symmetry; symmetry around the longitudinal plane).—This is the rule in the *Phænocystes*, but as no attention has been directed to the detection of asymmetry, it may be that it is present in a few species. It certainly forms a striking feature of *Myxobolus macrurus*, in which the differentiation of a dorso-ventral axis is perfectly plain. Further, the supero-median cornu extends farther forward than the inferior median cornu in several (all examined by me) *Myxobolus* species, furnishing another indication of this differentiation and a clue to the homology of the superior and inferior surfaces in different spores (see pp. 122, 235).

4. *Antero-posterior symmetry* (symmetry around the transverse plane). This type appears to be characteristic of, and confined to, the genus *Cystodiscus*, in which antero-posterior symmetry is equally present, whether we regard the extremities of the spores as (anterior and posterior) ends or as (right and left) wings.

The importance, for classification, of a study of spore symmetry is soon seen. Employing the knowledge thus obtained for the purpose of orienting the spore, we find that the characters of greatest taxonomic value are:

1. *Spore topography*.—Thus in *Myxidium lieberkühni* the presence of bilateral and the absence of antero-posterior symmetry show that the two pointed extremities of this spore, heretofore, like all other pointed extremities, loosely termed "ends," do not correspond to anterior and posterior, but to right and left. On the other hand the "ends" in *Cystodiscus* appear to represent ends *sens. strict.*, i. e., to correspond to anterior and posterior.

<sup>1</sup> With the further exception of two *Myxobolus* species (*M. unicusulatus* with only 1 capsule, and *M. inequalis* with 2 unequal capsules), which, on account of reduction of characters, have suffered a corresponding loss of the perfect symmetry characteristic of the genus. To make the exception absolutely complete, *M. strongylurus* may be added (see p. 249).

2. *Position and grouping of the capsules.*—Compared to these all-important characters, the mere number of the capsules is of minor importance. For, not only does the same genus frequently show 1 or 2, 2 or 4, but the number may even vary in the same species, as (apart from the entirely anomalous case of *Myxobolus ellipsoides*, where “accessory” capsules may develop) *Myxidium lieberkühni* shows sometimes 2 and sometimes 4 capsules.<sup>1</sup> But what is never varied in the same genus is the *topographic relation* of the capsules. Thus in *Myxobolus*, while in number they may be either 2 or 1, they are never arranged otherwise than in one group, or placed otherwise than at the anterior end, and similarly in all the other genera. In *Myxidium* the capsules are 2 or 4, but whether 2 or 4, they are always in two groups at the right and left extremities of the spore. Also in *Cystodiscus* they are 2 or 4, but always in two groups, which, however, are probably anterior and posterior in position (see p. 278).

In the following table I have plotted out the principal characters and indicated their relations to generic lines.

*Comparison of generic characters in the Phænocestes.*

[× = present; 0 = absent; ( ) = less usual; — = condition not known.]

	Symmetry.		Capsules.				Shell.		Vacuole.	Tail.	
	Antero-posterior.	Bilateral; perfect.	Number.	In one group (at the anterior end).	In two groups.		Inclination of plane of junction of valves to longitudinal plane.				
					At the (anterior and posterior) ends.	In the (right and left) wings.	Bivale.	0°.			90°.
<i>Myxobolus</i> Bütschli <i>sens. strict</i> .....	0	×	2 (or 1)	×	.....	.....	×	×	×	0	
<i>Henneguya</i> Thélohan.....	0	×	2	×	.....	.....	×	×	.....	×	
<i>Chloromyxum</i> Mingazzini.....	0	×	4	×	.....	.....	×	×	.....	0	
<i>Myxosoma</i> Thélohan.....	0	×	12	×	.....	.....	(?)	—	.....	0	
<i>Sphaerospora</i> Thélohan.....	0	×	12	×	.....	.....	×	—	.....	0	
<i>Ceratomyxa</i> Thélohan.....	0	+	12	×	.....	.....	×	—	.....	0	
<i>Cystodiscus</i> Lutz.....	×	×	2 (or 4)	.....	×	.....	×	.....	×	0	
<i>Sphaeromyxa</i> Thélohan.....	×	+	2	.....	(?)	.....	×	.....	×	—	
<i>Myxidium</i> Bütschli.....	0	×	2 (or 4)	.....	.....	×	0	.....	.....	0	

\* From analogy and general similarity of appearance, this genus can hardly be other than bivalve.

† *O. (S.) ohlmacheri*.

‡ Imperfect. Shell and capsules symmetrical; sporoplasm unilateral.

From this table we may conclude that—

1. *Henneguya* agrees with *Myxobolus* in every respect but one, the presence of a tail. (See also p. 206.)

2. Thélohan's groups, “*Myxidiées*” and “*Chloromyxées*,” must undergo rearrangement (see table below); for clearly *Chloromyxum*, *Myxosoma*, and *Sphaerospora* form a compact group, with which *Myxidium* has no character of consequence in common except the absence of a vacuole.

<sup>1</sup> Balbiani, 1883, Journ. de Microgr., VII, p. 274, fig. 64g.

3. *Sphaerospora* and *Myxosoma* do not differ at all in the characters given (the distinction between these unispecific genera resting solely upon the outline of the spore), and the two taken together present only a single character in contrast to *Chloromyxum*, viz, the number of the capsules. They may therefore be fused as a subgenus of *Chloromyxum*.

4. *Ceratomyxa* agrees sufficiently closely with *Chloromyxum* to permit its reference to the *Chloromyxidae*.

5. *Cystodiscus* is certainly entitled to separate family rank. To it may be provisionally approximated *Spheromyxa*, it having the capsules in two groups and a bivalve shell. (Compare carefully p. 278.)

6. *Myxidium* must form the type of a separate family, the entirely different position and grouping of the capsules forbidding its reference to the *Chloromyxidae*.

The following table shows the relations of Thélohan's classification to the one now proposed:

THÉLOHAN'S CLASSIFICATION.

No vacuole, 2 or 4 capsules.	{	2 capsules: Myxidians. Spores	Fusiform, 1 capsule at each extremity. <i>Myxidium</i> .
			Elongated; shell formed of two hollow-cone valves soldered along their bases. <i>Ceratomyxa</i> .
			Flattened-ovoid, more or less elongate. <i>Myxosoma</i> .
			Spherical. <i>Sphaerospora</i> .
		4 capsules: Chloromixans.....	<i>Chloromyxum</i> .
1 iodophilic vacuole; 1 or 2 capsules.	{	Myxobolans. Spore-shell	Destitute of a tail; capsules 1 or 2. <i>Myxobolus</i> .
			With a tail; capsules 2. <i>Henneguya</i> .

PROPOSED CLASSIFICATION.

GENUS.	FAMILY.	CHARACTERS.
<i>Myxidium</i> .....	<i>Myxididae</i> .....	Bilateral but not antero-posterior symmetry; capsules in two groups right and left; no bivalve shell; no vacuole.
<i>Ceratomyxa</i> .....	{ <i>Chloromyxidae</i> ...	Bilateral but not antero-posterior symmetry; capsules in one group (at the anterior end); a bivalve shell, with the valve-junction plane perpendicular to the longitudinal plane; no vacuole.
<i>Chloromyxum</i> , et subgen. <i>Sphaerospora</i> (including <i>Myxosoma</i> ).		
<i>Myxobolus</i> .....	{ <i>Myxobolidae</i> .....	Bilateral but not antero-posterior symmetry; capsules in one group (at the anterior end); a bivalve shell with the valve-junction plane parallel to the longitudinal plane; an iodophilic vacuole.
<i>Henneguya</i> .....		
<i>Cystodiscus</i> .....	{ <i>Cystodiscidae</i> .....	Bilateral and antero-posterior symmetry; capsules in two groups, anterior and posterior; a bivalve shell with the valve-junction plane perpendicular to the longitudinal plane; condition of sporoplasm unknown.
? <i>Sphaeromyxa</i> .....		

## SPECIFIC CHARACTERS.

**Spore-form:** This is a somewhat variable character, e. g., elliptic spores, varying in breadth; nevertheless, considerable dependence may usually be placed upon it.

**Tail:** I have elsewhere (p. 207) indicated my belief that the presence of a tail is a good specific character. The length of the tail relative to that of the body (caudal index) will also prove useful.

**Ridge index:** As the width of the ridge bears a very constant ratio to the whole width of the surface of which the ridge forms a part, this ratio is a good specific character, especially as it often differs markedly in different species.

**Capsular index:** This is a character of great constancy, and hence of much taxonomic value.

**Nuclei:** The presence or absence of the pericornual nuclei has proved constant in several species examined by me (see p. 210). The position of the remaining nuclei is inconstant.

## VI.—PATHOLOGY.

Pfeiffer says<sup>1</sup> that myxosporidian infection is characterized by the rapid disappearance of the nuclei of the infected cells, the infection of the red blood corpuscles, and the attacking of all the elemental tissues of the host, with the possible exception of those of the nervous system; further, through the early spore formation which is unconnected with any external evidence of maturity. And, further, considering how the blood parasites of *Emys*, *Lacerta*, birds, and of malarially diseased cattle and men, employ the blood-corpuscle membranes as protective coverings for their naked bodies; also, that the youngest myxosporidia, just out of the spore shell, attack the red blood corpuscles; and, further, that the *Myxosporidia* spare no organ or elemental cells (the nervous system possibly excepted), the destructiveness of this group of parasites must be recognized to be very great; and, further, that the parasite withdraws directly or indirectly a large quantity of blood from the host, is shown by the hæmatoidin crystals found in all myxosporidia. Finally, a cachexia, comparable with the cancerous cachexia of the warm-blooded animals, is produced.

By a reference to p. 187 it will be seen that Korotneff observed in the polyzoan, *Aleyonella fungosa*, substantially the same process that Pfeiffer records in *Lucius lucius*, viz, an intracellular development during the earlier myxosporidium stages.

*Mode of infection.*—Leydig<sup>2</sup> remarked that an organism like *Gen. incert. sp. 4.* could pass with the blood current into the various organs, effect a lodgment, become encysted, and give rise to the "psorosperms."

<sup>1</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., pp. 48-49; 2 ed., 1891, p. 135.

<sup>2</sup> Müller's Archiv, 1851, p. 229.

Lieberkühn<sup>1</sup> believed that such amœboid organisms attach themselves to the skin for the purpose of reproduction. Ludwig<sup>2</sup> thinks that the greater frequency of occurrence on the gills indicates a greater ease of infection through this channel than *via* the alimentary canal. Also he says:

The lymph channels of the connective tissue appear to represent the principal paths through which the parasite spreads itself further through the body.

He, however, fails to give any actual evidence in favor of this view. Pfeiffer<sup>3</sup> says:

The common occurrence of the *Myxosporidia* in all organs presupposes a distribution *via* the circulation, a mode demonstrated by the infection of the red blood corpuscles.<sup>4</sup>

*Effects.*—Upon this Balbiani<sup>5</sup> has the following:

Unlike the Gregarines and the *Coccidia*, the psorosperms spread themselves through almost all the organs, the deep as well as the superficial, the skin, spleen, kidney, air bladder, and even the heart and ovary. They are also found in the cells of the urinary tubules, and in the young Graafian follicles, which they transform into a pocket filled with psorosperms. As at the same time they increase with great rapidity, it results that animals thus infested present grave diseases and may even die. Certain morbid states of fish ought without doubt to be attributed to the *Myxosporidia*. Such is the case of that Merluce<sup>6</sup> observed by J. Müller and which was remarkable for an extraordinary emaciation. I have myself often seen roach, tench, and other fishes reduced by these parasites to a cachectic state characterized by a decoloration of the tissues, destruction of the red blood globules, and augmentation of the white globules; a veritable leucocythæmia. It is not, then, surprising that this disease can cause great ravages among fishes, above all in the young, which are most often affected. Nevertheless this cause is not usually noted as among those which destroy fishes. This is easily explained; when the disease reigns attempts are first made to explain it by macroscopic causes and ordinarily it is the worms which are accused. This was the case in the epidemic of the tench in the étangs of Dombes; it was the Ligules which interfered with digestion and the fishes died of inanition. Microscopic causes are not the ones most frequently suspected. I believe that more frequent search would reveal microscopic lesions capable of explaining the mortalities of young fish, particularly those living in marshes and in aquaria.

Upon this point M. Thélohan<sup>5</sup> remarks that these parasites are generally well borne, but that sometimes the tumors may cause death by pressure effects, e. g., he saw a cyst in *Gasterosteus aculeatus* produce fatal pressure upon the heart.

The principal extensive epidemics have been those involving the barbels and the crayfishes (see pp. 197, 231).

<sup>1</sup> Müller's Archiv., 1854, p. 357 (see also p. 185).

<sup>2</sup> Jahresber. d. rhein. Fisch.-Vereins, 1888, pp. 33-4.

<sup>3</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., p. 48.

<sup>4</sup> For the latter see p. 288.

<sup>5</sup> Journ. de Microgr., Paris, 1883, VII, pp. 280-281.

<sup>6</sup> I have elsewhere noted this error (p. 172). The fish in question is *Gadus morrhua* and not *Merluccius merluccius*.

<sup>7</sup> Annal. de Microgr., 1890, II, p. 203.

## VII.—MICROSCOPIC TECHNIQUE.

The older observers used no reagents beyond acetic acid, potassium hydrate, etc. Bütschli<sup>1</sup> was the first to use a staining reagent. He believed that alum carmine stained nuclei in the ectoplasm. The first observer to employ modern technique was Henneguy.<sup>2</sup> Subsequently Thélohan<sup>3</sup> employed similar technique, and Pfeiffer<sup>4</sup> devotes some space to the technique of protozoan investigation. Finally Henneguy and Thélohan<sup>5</sup> give a few additional remarks upon this subject.

The following is a summary of the methods recommended: Fixing and hardening preferably by chromic or osmic acid or both (Perenyi's or Flemming's liquids<sup>6</sup>) or corrosive sublimate solution. Washing out, dehydration, paraffining, sectioning as usual. Affixing to the slide by Mayer's albumen. Where alcohol-fixed material is the only kind available, much may be gotten out of it in the way of study of the spore.

Dissociation (1 per cent osmic acid solution; Ripart and Petit's liquid) shows certain facts better than the section method.

Sections are necessary to determine the seat, and, above all, to follow the different stages of development.

Culture in the blood (overhanging drop method) is recommended by Pfeiffer for the study of development.

Stains:<sup>7</sup> For alcoholic specimens, carmine; above all other forms hydrochloric acid alcohol carmine is very reliable. For chrom-osmium (and may be tried on alcoholic) specimens, especially gentian violet, double stain with the violet by eosin. Safranin, by Henneguy's method,<sup>8</sup> evinces an electivity valuable in the study of development where we have to do with the most complex phenomena of cellular life under circumstances in which the small size of the elements renders observation extremely difficult. The sections must be decolorized in clove oil for a very long time. Small stellate-grouped masses of crystals, which are often precipitated and whose presence is very annoying in the subsequent study of the section, may be easily removed by successive alternate washings of the latter in chloroform and bergamot oil.

Valve separation: Most certainly effected by sulphuric acid (cold, concentrated).

Vacuole: Best shown by very dilute iodine water (with potassium iodide).

<sup>1</sup> Ztschr. f. wiss. Zool., 1881, xxxv, p. 632.

<sup>2</sup> Mém. publiées Soc. philomat. Paris l'Occas. Centen. Fondation, 1888, p. 165.

<sup>3</sup> Annal. de Microgr., 1890, II, p. 196.

<sup>4</sup> Die Protozoen als Krankheitserreger, 1891, 2 ed., pp. 19-24.

<sup>5</sup> Annal. de Microgr., 1892, IV, pp. 620-621.

<sup>6</sup> Also Kleinenberg's liquid (Henneguy, 1888).

<sup>7</sup> Henneguy (1888) also used picrocarmine.

<sup>8</sup> Journ. Anat. et Physiol., Paris, 1891, xxvii, pp. 398-400.

Filament extrusion: Most certainly produced in the fresh state by strong sulphuric acid, iodine water, glycerin, nitric, hydrochloric, acetic, formic acids, alkaline hydrates, boiling water, ether, etc., especially the first two. In alcoholic specimens, also, occasional spores extrude their filaments under the action of sulphuric acid or iodine.

#### VIII.—DEFINITIONS.

Anterior (and posterior): There can be no question that the longitudinal diameter is the antero-posterior axis of the body. The discrimination of anterior from posterior is, however, in the absence of cephalization, impossible. I have followed custom in calling the sharper, capsular end "anterior," and the opposite rounded end "posterior."

Capsules: The pyriform, hollow, filament-containing bodies characteristic of the myxosporidian spore ("twinned vesicles" of Balbiani; "polar capsules" of Bütschli). "Capsule" is preferred to "vesicle" on account of greater definiteness, and to "polar capsule," as the situation implied by the latter is not constant.

Cornua: The pointed anteriorly projecting extremities of the sporoplasm. They are infero-, and supero-lateral, and infero-, and supero-median. (See also *Surface, superior*, p. 122.)

Diameter, longitudinal: The line formed by the intersection of the longitudinal and vertical planes.

Diameter, transverse: The line formed by the intersection of the transverse and longitudinal planes.

Diameter, vertical: The line formed by the intersection of the vertical and transverse planes.

Ducts: The ducts into which the capsule is drawn out anteriorly and which serve for the exit of the filaments.

Ends (of the spore): The median (anterior and posterior) extremities in contradistinction to the wings.

Filaments: The filaments which lie coiled within the capsules. The "capsular filaments," "spiral filaments," and "coiled filaments" of the authors. *Not* to be confounded with the ribbonettes.

Host: In the usual sense; see also *Seat*.

Myxoplasm: The protoplasm of the myxosporidium.

\*Myxosporidium: The amœboid adult stage; *Mutterblase*, Leydig.

Pansporoblast: see *Sporoblast*.

Pericystic space: The space apparently empty (presumably fluid-filled) surrounding the capsules.

Plane, longitudinal:<sup>1</sup> Horizontal and percapsular, passing through both capsules and the sporoplasm, and dividing the spore into a superior and an inferior portion.

<sup>1</sup> For brevity and clearness these planes are defined as if rectangularly arranged about the center of the *Myxobolus* spore, the latter being supposed to be viewed "on the flat."



Plane, transverse:<sup>1</sup> Vertical and (usually) post-capsular in position, dividing (roughly) the spore into a capsular (anterior) and a sporoplasmic (posterior) portion.

Plane, vertical: Longitudinal and intercapsular, passing between the capsules and through the ends of the spore and the median cornua of the sporoplasm, and dividing the spore into a right and a left half.

Posterior: See *Anterior*.

Protoceysts: The two smaller segments of the *Myxobolus* sporoblast, which ultimately form the capsules.

Protoporoplasm: The larger segment of the *Myxobolus* sporoblast, which ultimately forms the sporoplasm.

Ribbon: The shell processes described by Balbiani in *Myxobolus ellipsoides* (see pp. 223).

Ribbonettes: The terminal subdivision of the ribbons, termed "filaments" and confounded with the capsular filaments by some writers (see pp. 87, 88, 263).

Ridge: The ridge or "welt" which extends around the circumference, and marks the line of junction of each valve.

Ridge index: The ratio of the width of the ridge to the total width of the surface on which the ridge is situated.

Seat: This term invariably denotes the organ or part of the body in which the myxosporidian is located (see also *Host*).

Sporoblast (and pansporoblast): This term was first used (in the *Myxosporidia*) by Bütschli<sup>2</sup> for the transparent spherical globule formed by the condensation around one of the nuclei, of a portion of the surrounding myxoplasm. The spherical globule so formed subsequently segments into two hemispheres (see p. 81), each of which gives rise to a spore. Now, Balbiani,<sup>3</sup> and Thélohan,<sup>4</sup> and Henneguy and Thélohan,<sup>5</sup> apply the term sporoblast to the two hemispheres. Further, Pfeiffer<sup>6</sup> uses the term sporoblast as a synonym for the *whole* sporing *myxosporidium*. This latter use of the word should, I think, be unhesitatingly rejected as having no warrant in analogy. By the advice of Dr. C. W. Stiles (who has specially studied the equivalence of this and several other terms<sup>7</sup>), I have followed the lead of Balbiani and Thélohan in restricting the term *sporoblast* to the segments (the two hemispheres above mentioned) formed by the division of the primitive sphere. For the latter (the sporoblast of Bütschli) the term *pansporoblast* is here used.

<sup>1</sup> Equatorial plane of Lutz, 1889, Centralbl. f. Bakt. u. Parasitenkde, v, p. 86.

<sup>2</sup> Bronn's Thier-Reich, 1882, I, p. 596. He says: "Since the spores originate from the plasma globules, we may conveniently term them *sporoblasts*." Compare also an exceedingly obscure sentence in Bütschli's next paragraph.

<sup>3</sup> Journ. de Microgr., Paris, 1883, VII, p. 275.

<sup>4</sup> Compt. Rend. Acad. Sci. Paris, 1890, CXI, p. 693.

<sup>5</sup> Annal. de Microgr., Paris, 1892, IV, p. 634.

<sup>6</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., pp. 32, 34, *et al.*

<sup>7</sup> Notes on Parasites; Journ. Compar. Med. & Veter. Archives, New York, 1892, XIII, pp. 321-324.

Sporocyst (rejected): Synonym for spore. Employed by Pfeiffer.<sup>1</sup>

Sporoplasm: The "posterior mass," "plasmic mass," etc., of the spore. This term is used as the equivalent of the phrase "protoplasm of the spore."

Surface, inferior: That upon which the inferior valve (*q. v.*) and the infero-median cornu are situated (see also next).

Surface, superior: That upon which the superior valve (*q. v.*) and the supero-median cornu are situated.

These are, respectively, the equivalent of dorsal and ventral, or of ventral and dorsal. In the absence of hæmal and nervous systems and of an alimentary tract, the proper correlation of these surfaces with the corresponding ones in extra-myxosporidian organisms seems impossible. *Inter se*, however, the superior surfaces may be correlated by a greater convexity of the superior valve, but probably most frequently by the *further projection forward of the supero-median cornu*, which may (?) even reach the extreme anterior end of the shell cavity.

Valve: Each shell half.

Valve, inferior: The less convex valve; see also next.

Valve, superior: The more convex valve. The differentiation is probably possible in only a few cases. The supero-median cornu will probably form a better guide to the discrimination of the superior and inferior surfaces.

View, longitudinal, transverse, or vertical; view along the line of the corresponding diameter (*q. v.*).

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<sup>1</sup> Die Protozoen als Krankheitserreger, 1891, 2 ed., pp. 7, 8.

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<sup>1</sup> Pfeiffer, in his bibliography of the *Myxosporidia* (Ztschr. f. Hygien., 1888, IV, p. 436), erroneously includes with the word "Myxosporidia" the following: Blanchard, Bull. Soc. Zool. France, 1885, X, p. 291. The citation is an error and Blanchard's only paper in the volume (pp. 244-276) is sarcosporidian.

<sup>2</sup> According to Pfeiffer (Ztschr. f. Hygien., 1888, IV, p. 436) these 3 articles are the same. They are here given separately on account of the different titles shown by the Royal Society's Catalogue.

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<sup>1</sup> Misquoted by Pfeiffer (1890, *infra*, p. 49) as "Bacteriologisches Centralblatt, 1890."

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<sup>2</sup>Misquoted "d'Agricult." (Pfeiffer, 1891, *infra*, p. 105.)

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LXXIV.

LXXV.

LXXVI.

LXXVII.

LXXVIII.

LXXIX.

LXXX.

LXXXI.

LXXXII.

LXXXIII.

LXXXIV.

LXXXV.

LXXXVI.

LXXXVII.

LXXXVIII.



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*b. The literature by authors.*

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Do .....	1866	XXII.	Lieberkühn .....	1854	XVI.
Do .....	1867	XXIII.	Do .....	1854	XVII.
Do .....	1882	XXXV.	Do .....	1854	XVIII.
Do .....	1884	XXXVI.	Do .....	1855	XIX.
Bessels .....	1867	XXIV.	Linton .....	1891	LXX.
Borne .....	1886	XLVII.	Do .....	1891	LXXI.
Braun .....	1893	LXXXIV.	Ludwig .....	1888	LIV.
Bütschli .....	1881	XXXII.	Lutz .....	1889	LV.
Do .....	1881	XXXIII.	Mégnin .....	1885	XXXIX.
Do .....	1882	XXXIV.	Do .....	1885	XL.
Claparède .....	1874	XXV.	Mingazzini .....	1890	XLV.
Creplin .....	1842	VI.	Moniez .....	1887	XLIX.
Dujardin .....	1845	X.	Müller .....	1841	III.
Engler & Prantl .....	1892	LXXXIX.	Do .....	1841	IV.
Gabriel .....	1880	XXXI.	Do .....	1841	V.
Garbini .....	1891	LXIX.	Do .....	1843	VIII.
Gluge .....	1838	I.	Ohlmacher .....	1893	LXXXVI.
Do .....	1841	II.	Perrier .....	1893	LXXXIII.
Gurley .....	1893	LXXXVII.	Perugia .....	1890-91	LXVII.
Heckel & Kner .....	1858	XX.	Pfeiffer .....	1887	L.
Henneguy (see also Hen- neguy & Thélohan; Thé- lohan & Henneguy) .....	1888	LII.	Do .....	1888	LI.
Do .....	1889	LVI.	Do .....	1890	LXII.
Henneguy & Thélohan .....	1892	LXXXVIII.	Do .....	1890	LXIII.
Do .....	1892	LXXXII.	Do .....	1891	LXXII.
Kner (see Heckel & Kner).			Do .....	1893	LXXXVIII.
Koch .....	1887	XLVIII.	Prantl (see Engler & Prantl)		
Kolesnikoff .....	1886	XLII.	Railliet .....	1886	XLIII.
Korotneff .....	1892	LXXIV.	Do .....	1886	XLVI.
Kruse .....	1892	LXXXVI.	Do .....	1890	LXIV.
Ladague .....	1884	XXXVII.	Do .....	1893	XCI.
Lankester .....	1885	XLI.	Rayer .....	1843	VII.
Leclercq .....	1890	LXI.	Do .....	1843	IX.
Leuckart .....	1852	XIV.	Remak .....	1852	XIII.
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Do .....	1886	XLIV.	Schneider .....	1875	XXVII.
Leunis .....	1886	XLV.	Sibley .....	1890	LX.
Leydig .....	1851	XI.	Solger .....	1877	XXVIII.
			Sticker .....	1892	LXXXIX.

b. *The literature by authors*—Concluded.

Author.	Date.	Bibliographic reference number.	Author.	Date.	Bibliographic reference number.
Thélohan (see also Henneguy & Thélohan; Thélohan & Henneguy).....	1889	LVII.	Thélohan (see also Henneguy & Thélohan; Thélohan & Henneguy).....	1893	LXXXV.
Do.....	1890	LVIII.	Do.....	1894	XCII.
Do.....	1890	LIX.	Thélohan & Henneguy.....	1892	LXXXVII.
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Do.....	1892	LXXXIII.	Whinery.....	1893	XC.
Do.....	1892	LXXX.	Wittmack.....	1875	XXVI.
Do.....	1892	LXXXII.	Zschokke.....	1884	XXXVIII.

TABLE SHOWING THE DERIVATION AND EQUIVALENCE OF ALL FIGURES IN THIS PAPER REPRODUCED FROM PREVIOUS AUTHORS.

The following table shows the equivalence of all figures in the literature, including those of species formerly considered myxosporidian but now rejected. Figures to the right are copied from those farther to the left on the same horizontal line, and those copied in this paper are, in all cases, taken directly from the original. Further, wherever several series of letters or figures (indicated, for economy of space, as "*a-m*" "1-16, etc.) occur on the same horizontal line, the individual members of such series correspond *always and rigidly* each to each, that is, *a* to *a*, *b* to *b*, 1 to 1, 2 to 2, or 7 to 10, 8 to 11, etc., as the case may be. To save space all intermediate columns not required on any particular page are omitted from that page. Such omitted columns will of course appear on some other page, and their relative positions in the full series of illustrated articles represented in this table, are indicated by the bibliographic reference number (Roman numerals). Plate numbers (heavy type) are inserted only where absolutely necessary to prevent ambiguity.

After much study of the literature certain figures can not now be placed with any certainty. They are those to which no species number corresponds in the table. It will be seen that they are principally some of Pfeiffer's and Balbiani's and are mainly to be distributed between the two probably very distinct but at present not very clearly delimited species habitant on the tench, *Myxobolus piriformis* and *M. ellipsoides*. On the plates I have thought it best to reproduce the groups of figures entire and to leave to the future the apportionment of the individual figures, and will only add that in the synonymy of *M. piriformis* and *M. ellipsoides* I have ventured on a taxonomic guess, the dubious figures being separated from those definitely placed by a period or a parenthesis.



Table of equivalence of figures—Continued.

Lieberkühn, 1854, XVI.	Lieberkühn, 1855, XIX.	Balbani, 1867, XXIII.	Leuckart, 1879, XXIX.	Ryder, 1880, XXX.	Bütschli, 1881, XXXIII.	Bütschli, 1882, XXXIV.	Zschokke, 1884, XXXVIII.	Lankester, 1885, XLI.	Leuckart, 1886, XLIV.	Leunis, 1886, XLV.	Koch, 1887, XLVIII.	Engler & Prandl, 1892, LXXIX.	Gurley, 1894.	Species No.
21-27													14, 4a-g	38
28													7, 4a	13
1													43, 1a	100
2													46, 3	100
3				98					98		668, 1		47, 2	100
4													39, 5a	96
5				99a		17			99A		668, 3		15, 7b	96
6						18b					668, 2		7, 4b-e	44
7				99b		18c			99B				5, 2-4	44
8													6a-c	13
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	1												12	41
	6-8												13a-c	41
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		11											4b-d	98
		12a-c											17, 1a	98
				1A-C									16, 3a	98
				2B-D									17, 5	74
					1								16, 6a	74
					2								17, 7a	46
					3	6b		40					16, 5	46
					4								16, 1	46
					5	7							44, 1a	100
					6	8							47, 3	100
					7								44, 4	100
					8								43, 3	100
					9								44, 3	100
					10								47, 5	100
					11								44, 5a-d	100
					12								47, 1a	100
					13								47, 1b	100
					14	10a							22C	100
					15	9		41					22D	100
					16								40, 7	100
					17								43, 5	92
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					20								15, 7a	80
					21								19, 1	80
					22	6a				1, 118			6, 1	80
					23								36, 4	44
					24								40, 3	48
					25								40, 3	9
					26								14, 7a-b	83
					27								6, 2	91
					28								31, 1a-e	40
					29									10
					30									68
					31									
					32	15								
					33									
					34a-d									
					35	14a								
					36	b								
					37	c								
					38	d								
					39									
					40									
						5								
						11								
						12		34						
						13								
						16a		43						
						b								
						c		44						
						18a								
						19								
						20								
						21		42						
						22								
						23a-b								
						24								
							16							

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Balbani, 1883, XXXV.	Balbani, 1884, XXXVI.	Mégnin, 1885, XXXIX.	Kolesnikov, 1886, XLII.	Railliet, 1886, XLVI.	Borne, 1886, XLVII.	Pfeiffer, 1887, L.	Pfeiffer, 1888, LI.	Henneguy, 1888, LII.	Ludwig, 1888, LIV.	Lutz, 1889, LV.	Henneguy, 1889, LVI.	Thélohan, 1890, LVIII.	Pfeiffer, 1890, LXII.	Pfeiffer, 1890, LXIII.	Pfeiffer, 1891, LXII.	Gurley, 1894.	Species No.
40a-h	36a-h			72a-h							2a-h					21, 1a-h	49
41a-d	37a-d															34, 3a-d	80
42a-d	38a-d															4a-d	80
43a-b	39a-b															28, 7a-b	57
44a-b	40a-b															40, 2a-b	90
45a-c	41a-c															39, 6a-c	96
61a-e	42a-e															21, 2a-e	49
62a-c	43a-c						15a									20, 1a-c	49
63a-c	44a-c															2a-c	49
64a-d	45a-d															46, 2a-d	100
64a-g	45e-g															47, 4a-c	100
65a-c	46a-c															20, 3a-c	49
66a-f	47a-f															13, 4a-f	(*)
	1															19, 4	49
	2															18, 1	35
	3A															2	49
	B															13, 3A	(*)
	C															B	(*)
		A														C	(*)
	A'-A'''															25, 1a	51
	B-H															a <sub>1</sub> -a <sub>2</sub>	51
																b-h	51
																35, 1	81
																2-6	81
																7	81
																29, 8a-b	67
																22, 1	(*)
																2	(*)
																3	(*)
																21, 4	(*)
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																25, 2a-c	51
																42, 1	97
																2	97
																3	97
																4	97
																5	97
																6	97
																7	97
																8	97
																9	97
																10	97
																1	78
																31, 2	88
																19, 5	49
																6	49
																10, 2	28
																34, 2a	80
																b	80
																c	80
																14, 8a	42
																b	42
																c	42
																d	42
																20, 4a	49
																b	49
																c	49
																d	49
																e	49
																10, 3a-i	28
																31, 4	71
																7, 5	(f)
																25, 3	51
																24, 1a-h	51
																24, 2a-e	51
																46, 1a-b	100
																45, 3a-c	100
																19, 7, 8	49
																45, 1a-h	100
																21, 3	(*)
																23, 2a-d	51
																45, 2 I	100
																IIa-b	100
																IIIa-b	100
																IV	100
																25, 4	51
																5a-c	51

\* See pp. 211, 294.

† Sarcosporidian falciform body from the sheep.

Table of equivalence of figures—Continued.

Perugia, 1891, LXXVII.	Garbini, 1891, LXIX.	Linton, 1891, LXX.	Linton, 1891, LXXI.	Korotheff, 1892, LXXIV.	Weltner, 1892, LXXV.	Théloban, 1892, LXXX.	Cuénot, 1892 (see p. 171).	Honnegny & Théloban, 1892, LXXXI.	Schewiakoff, 1893 (see p. 175).	Oltmacher, 1893, LXXXVI.	Préifier, 1893, LXXXVIII.	Stiles, 1893 (see p. 175).	Whinery, 1893, XC.	Gurley, 1894.	Species No.
1-6														37, 12-7	94
7-8														14, 5-6	39
9-14														29, 12-7	64
15-20														6, 3-8	11
	a													12, 3 a	33
	b													b	33
	c													c	33
	d													d	33
	e													e	33
		1-16												27, 1-16	55
			1											7, 1	12
			2a											a'	12
			b											b	12
			b'											b'	12
			c											c	12
			3											3	12
				1										8, 1	26
				2										2	26
				3										3	26
				4										4	26
				5										9, 1a	26
				6										1b	26
				7										1c	26
				8										1d	26
				9										1e	26
				10										2	26
				11										3	26
				12										4	26
					1-16									30, 1a-g	69
						1								41, 4	87
						2								39, 4	95
							1							2, 1	2
							2a							2a	2
							b							b	2
								1						11, 1	31
								2						2	31
								3						3	31
								4						4	31
								5						5a	31
								6						b	31
								7						c	31
								8						d	31
								9						12, 1	32
								10-20						2a-l	32
								21						m	32
								22						n	32
								23						o	32
								24						p	32
								25a-b						q, r	32
								26						10, 4	30
								27a-b						5a, b	30
									17-23					4, 2-8	6
									24-32					5, 1-9	6
									33a-l					10a-l	6
									34a-b					11a-b	6
										1				40, 8	89
										2				41, 1a	89
										3				2	89
										4a				1b	89
										b				c	89
										c				d	89
											13			22, 5	51
											14			6	51
											15			23, 1	51
											16			25, 6	51
												1		3, 1	5
												2		2	5
												3		3	5
												4		4	5
												5a		5a	5
												b		b	5
													1	41, 3	89

## DESCRIPTION OF GENERA AND SPECIES.

*Tabular Key.*

The following tabular key includes all the species, which can by any reasonable possibility be construed as myxosporidian, with their principal characters plotted out. The order of arrangement is a trifle more artificial than that found in the text.

Descriptions of the following species are omitted, as I believe there is no rational chance of their being *Myxosporidia*:

*Psorospermium hückelii* Hilgendorff, 1883.

(Parasite of *Astacus fluviatilis*, Hückel, 1855, *De telis quibusdam Astaci fluviatilis*, Inaug. Dissert. Friedr. Wilhelm. Univ. Berlin, p. 42, pl. 2, fig. 25A-C; *ib.* Hückel, 1857, Ueber d. Gewebe d. Flusskrebses, Müller's Archiv., pp. 561-2, pl. 19, fig. 25A-C; *ib.*, Grobben, 1878, Beiträge z. Kenntn. d. männl. Geschlechtsorg. d. Dekapoden; not seen.

*Psorospermium hückelii*, Bericht d. Gesellsch. Naturf. Freunde Berlin, pp. 179-181 (not seen); *ib.*, Zacharias, 1888, Ueber *Psorospermium hückelii*, Zoolog. Anzeiger, XI, pp. 49-51 (abstr. Journ. Roy. Micr. Soc. London, 1888, VIII, p. 240); *ib.*, Wierzejski, Kleine Beiträge z. Kenntn. d. *Psorospermium hückelii*, Zoolog. Anzeiger, XI, pp. 230-231 (abstr. Jour. Roy. Micr. Soc. London, 1888, VIII, p. 598).

This form and the next have never been definitely referred to the *Myxosporidia*, but Prof. Linton's bibliography of the "*Psorospermia*"<sup>1</sup> includes the articles containing them. They have no connection with the *Myxosporidia*.

*Psorospermium lucernariae* Vallentin, 1888.

Zoolog. Anzeiger, XI, pp. 622-623; abstr. Journ. Roy. Micr. Soc. London, 1889, pp. 75-76.

See note on preceding.

Pfeiffer<sup>2</sup> states that *Myxosporidia* were found by Leuckart and Lieberkühn in the gall bladder and the kidneys of toads. Now, the assertion, in so far as it concerns Leuckart, is, I suspect, an error. It was probably copied from Lutz,<sup>3</sup> who says:

The *Myxosporidia* are, as it is known, entirely parasitic, and in the large majority of cases live upon fishes. The only one of the authors accessible to me who mentions their occurrence in the *Amphibia* is Leuckart, who found them frequently in the urinary bladder of frogs, and also mentions the occurrence of a species described by Lieberkühn in the kidney.

I have been unable to find any such observation of Leuckart's, and correspondence with both him and Dr. Lutz failed to elicit a reference or a substantiation of the statement; so that "Leuckart" is here probably an error for Lieberkühn. Furthermore, there is absolutely nothing to indicate the myxosporidian nature of the forms described by

<sup>1</sup> Bull. U. S. Fish Com. for 1889, IX, p. 102.

<sup>2</sup> Virchow. Archiv. f. pathol. Anat. u. Physiol., Berlin, CXXII, p. 557; Die Protozoen als Krankheitserreger, 1891, 2 ed., p. 134; recently copied by Ohlmacher, Journ. Amer. Med. Assoc., 1893, XX, p. 562.

<sup>3</sup> Centralbl. f. Bakt. u. Parasitenkde, 1889, v, p. 84.

Lieberkühn.<sup>1</sup> On the contrary, both his descriptions and figures (which show spores, apparently of two different species, containing falciform corpuscles) justify the opposite conclusion. And Lankester<sup>2</sup> distinctly affirms its coccidian nature.

Possibly, Pfeiffer<sup>3</sup> says, a form reported by Kunstler and Pitres<sup>4</sup> from a pleural exudate of man is perhaps referable here. But from their descriptions and figure it is hard to see how by any possibility it could belong to the *Myxosporidia*. The smallest spores are 18  $\mu$  "long" and the largest 100  $\mu$ . In such large spores it is inconceivable that the capsules could be missed, and Kunstler and Pitres appear to regard it as coccidian.

Further, Pfeiffer says:

Also relations exist with a form found in chickens by Arloing and Tripier.

The following data will suffice for its rejection:

Arloing and Tripier<sup>5</sup> tell us that they found oval bodies with granular contents, a clear central nucleus, and a sort of "button" at each extremity of the longer diameter. These bodies measure 500 to 550  $\mu$  (400 to 450  $\mu$ , excluding the "buttons") in length, and 200 to 220  $\mu$  in breadth. Balbiani, from an examination of hardened specimens, reserved his opinion, but rather believed them to be "psorosperms." In spite of and after this, the authors tell us that they identified these oval bodies by finding *identical bodies in the oviduct of a worm* found imbedded in the same situation (oesophageal mucosa); in other words, they are the ova of a worm. It is hardly necessary to go further than their dimensions to exclude them from the possibility of being myxosporidian spores. It might, however, be added, that Balbiani would certainly have noted in his *Lçons sur les Sporozoaires* (1884) such an unprecedented anomaly as the occurrence of a myxosporidian in a bird.

I cannot, perhaps, better place the following remarks made by M. Armand in the way of discussion of Arloing and Tripier's paper. M. Armand, in concert with Balbiani, undertook, in 1873, the inoculations of "psorosperms" both in warm and in cold blooded animals. The attempt succeeded, and several pieces showing the proliferation and modifications of these bodies transported into organisms very different from their normal habitat were obtained, and preserved in the collection of the Laboratory of General Physiology of the Jardin des Plantes. As the subsequent myxosporidian literature is silent upon this point, it is probably safe to presume either that in this case "psorosperms" did not mean *Myxosporidia*, or, if it did, that the myxosporidian branch of the work proved barren of results.

<sup>1</sup> Müller's Archiv., 1854, pp. 1-5, pl. 1, figs. 1-19.

<sup>2</sup> Encyclop. Britan., 9 ed., XIX, 1885, p. 855.

<sup>3</sup> Die Protozoen als Krankheitserreger, 1 ed., 1890, p. 49; 2 ed., 1891, p. 135.

<sup>4</sup> Sur une psorospermie trouvée dans une humeur pleuritique; Journ. de Microgr., 1884, VIII, pp. 469-474, 520-526, pl. 11, figs. 1-15; pl. 12, figs. 1-3.

<sup>5</sup> Lésions organiques de nature parasitaire chez le poulet; Compt. Rend. Assoc. franç. l'Avanc. Sci., 1874, 2d (Lyons) Sess., pp. 810-814.



Parasite of *Syngnathus*, Pfeiffer, 1891, Die Protozoen als Krankheitserreger, 2 ed., p. 111, figs. 46-49:

From a perusal of the description and an examination of the figures I can find no evidence of myxosporidian affinities, and have therefore excluded this form. While this paper is passing through the press, I have, however, observed Pfeiffer's paper,<sup>1</sup> in which, in the portion devoted to the *Myxosporidia*, he says:

Of the *Syngnathus* from the North Sea, which the author was able to investigate two years ago in Helder (Holland), the relative conditions have been thoroughly pictured by the author in another place.

Finally, a comparison with the following may perhaps not be inadvisable:

Csokor, Gregarinosi d. Forellen, Oesterreich. Ztschr. f. wiss. Veterinärkde, Wien, 1888, II, pp. 56-58.

The author says the forms observed were undoubtedly referable to the "oviform and glöbular Coccidia (Gregarines)." From the general tenor of his description I suspect they were not *Myxosporidia*, and in any case there is at present no evidence to warrant their admission into the subclass.

Hardly any explanation of the table is necessary. The grouping and position of the capsules (and the correlated orientation of the spore) is made the leading character. Next come the other generic characters (bivalve condition of shell, presence or absence of vacuole, etc.).

One of the most important uses of this table is to direct attention to the gaps in our knowledge. Thus it will serve a useful purpose in showing readily where work is most needed.

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<sup>1</sup> Centralbl. f. Bakt. u. Parasitenkde, 1893, XIV, p. 124.

## Tabular key.

Species No.	Genera.	Spore.							
		Capsules.				Shell.		Vacu- ole.	Symmetry.
		1 only.		2 or more in—		Bivalve.	Inclination of valve-junction plane to longitudinal.	Antiodinophile.  Iodinophile.	Antero posterior.  Bilateral
		Obscure; spore minute. Conspicuous; spore relatively large.		1 group (at anterior end).	2 separated groups. At each (anterior and posterior) end. In each (right and left) wing.				Supero inferior.
1	Psorospermia (non-myxosporidian).					Operculate			Obscure or absent.
2	Lithocystis (non-myxosporidian).								
3	Genus incert. (non-myxosporidian).					Valves in contact at middle, diverging towards their ends.			Apparently symmetrical in all directions.
4	.....do.....								
5	Balbiania .....								
6	Genus incert. (non-myxosporidian).					Apparently 0.		Apparently none.	
7	Genus incert. (probably myxosporidian; spores unknown).								
8	.....do.....								
9	.....do.....								
10	.....do.....								
11	.....do.....								
12	.....do.....								
13	Genus incert. (aberrant <i>Myxosporidia</i> ?).								

*Tabular key.*

[illegible]

## Tabular key.

Species No.	Myxosporidium.							Cyst.			Host.
	Size in $\mu$ .	Ectoplasm and endoplasm differentiated.	Pseudopodia.	Nuclei.	Pansporoblast.	Pansporoblast producing spores <i>ad plurimum</i> .	Vacuole.	Size in mm.	Shape.	Color.	
1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	Sciæna umbra .....
2	Gregarine ovoid, 65 $\mu$ long, with a very voluminous nucleus and clinorhombic crystals.							1 to 2 in diameter.	Spherical; containing spores and rest of segmentation.	-----	Echinocardium cordatum.
3	Spores develop endogenously, several within a globule, the latter sometimes with an enveloping membrane; spores at first unsplit.							-----	-----	-----	Gadus morrhua .....
4	Amœboid mass 7.5 to 12 $\mu$ , with blunt processes and sometimes a tail; ends clear, center containing many dark corpuscles.							-----	-----	-----	Salmo fario .....
5	-----	-----	-----	-----	-----	-----	-----	2 to 4 by 0.7.	Oval.	White.	Anas bosch .....
6	7 by 3 to 20 by 6; plasmodies 18 by 8 to 48 by 23.	Probably lobulate, hyaline.	Blunt, lobulate, hyaline.	1 globular.	Apparently absent (cf. p. 179.)	-----	contractile, postero-peripheral.	Diameter 10; plasmodies 30-60; elongate tubes 70 by 24.	Globular.	Dark.	Cyclops (in part... lar <i>C. strenuus</i> ), <i>Diaptomus ceruleus</i> , <i>D. richardi</i> .
7	Small and granule-free to larger and granular; processes rather sharp; in size not equal to a blood corpuscle of the fish; granules extremely small, held together by a mucoid substance.							-----	Present; membrane transparent.		Abramis brama .....
8	Consisting of granular protoplasm; very similar to a <i>Chloromyxum mucronatum</i> ; apparently very variable, oval, lenticular, or dendroidly branched; size 27 to 440 $\mu$ ; with or without a structureless membrane; pansporoblast bisporogenetic.							-----	Apparently no true cyst.		Perca fluviatilis .....
9	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	Lota lota .....
10	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	Lota lota .....
11	Form variable, movements incessant, slow, amœboid.							-----	-----	-----	Leptocephalus conger.
12	-----	-----	-----	-----	-----	-----	-----	2.5 to (clusters) 7 by 5.	-----	-----	Notropis megalops.
13	-----	-----	-----	-----	Present??	-----	-----	1.09 to 2.18 by 0.44.	Cylindrical, rarely ellipsoidal or spherical.	-----	Gasterosteus aculeatus.

## Tabular key.

Seat.	Pathologic effect.	Remarks.	Genus.	Species.	Species No.
Dissection of abdominal septum.		Spore leathery, contents granular, colorless, amber or fuscous yellow, forming indefinite cylindrical, filamentous or spiral colonies; rarely isolated.	Psorospermia...	sciænæ-umbræ...	1
Body cavity; digestive tube.		A perfectly typical monocystid Gregarine. Gregarine stage passed usually in digestive tube. Spores contain 8 falciform corpuscles.	Lithocystis....	schneideri.....	2
Air bladder.....	Atrophy of tail muscles.	Pathologic mass whitish-yellow, pasty, drawing out into dirty white threads.	Genus incert...	sp. incert.....	3
Blood.....			do.....	sp. incert.....	4
Interstices of muscles.			Balbiana.....	rileyi.....	5
Body cavity, abdomen, thorax, tail, natatory feet, first antennæ.			Genus incert...	sp. incert.....	6
Branchiæ; ? also of heart blood.			Genus incert....	sp. incert.....	7
Branchiæ.....			do.....	sp. incert.....	8
Gall bladder.....			do.....	sp. incert.....	9
Branchiæ.....			do.....	sp. incert.....	10
Gall bladder.....			do.....	congru.....	11
Subcutaneous tissue.			do*.....	sp. incert.....	12
Subcutaneous tissue.		Spore containing a central globule ("nucleus") 7 to 11 $\mu$ in diameter, surrounded by several fine granules.	Genus incert....	sp. incert.....	13

\* "Myxosporidium," name not in good standing, see p. 206.

## Tabular key—Continued.

Species No.	Genera.	Spore.							
		Capsules.				Shell.		Vacu- ole.	Symmetry.
		1 only.		2 or more in—		Bivalve.	Inclination of valve-junction plane to longitudinal.	Anidnophile.  Iodinophile.	Antero-posterior.
		Obscure; spore minute. Conspicuous; spore rela- tively large.		1 group (at anterior end).	2 separated groups. At each (anterior and posterior) end. In each (right and left) wing.				
14	Genus incert. (most possibly all, myxo- sporidian).								
15	do			Capsules 2					
16	do								
17	do								
18	do								
19	do								
20	do								
21	do								
22	do								
23	do								
24	do								
25	do								
26	"Myxosporidium"					0		2 "vac- uoles."	
I	Glugea	×				0		×	
27	do	×							0
28	do	×				0		×	0
II	Pleistophora	×				0		×	0
29	do	×						×(?)	
III	Thelohania	×				0		×	0
30	do							×	0

## Tabular key—Continued.

Spore.															
Tail.				Outline on vertical view.	Dimensions.					Index.					
Absent.	Present.				Length in $\mu$ .	Breadth in $\mu$ .	Thickness in $\mu$ .	Length of capsules in $\mu$ .	Presence of filament; length in $\mu$ .	Capsular.	Caudal.	Ridge; presence and index.			
	Undivided.	Single.	More or less bifurcate.												
													Double from base.		
×														14	
									Very long; extruded by glycerin.						15
															16
															17
															18
															19
															20
															21
															22
															23
															24
															25
															26
0				Elongate-oval; sharp anteriorly, rounded posteriorly.											I
3															27
0				Regularly ovoid.	3 to 5	2 to 3.			50; extruded by iodine only.				4		28
0															II
				Ovoid.	3	1.5 to 2.0			Present.					Chromatophile bodies 4.	29
0															III
0				Ovoid.	2 to 3										30

## Tabular key—Continued.

Species No.	Myxosporidium.						Cyst.			Host.
	Size in $\mu$ .	Ectoplasm and endoplasm differentiated.	Pseudopodia.	Nuclei.	Pansporoblast.	Pansporoblast producing spores ad plurimum.	Size in mm.	Shape.	Color.	
14						2 or 3				Leuciscus cephalus.
15										Leuciscus cephalus.
16										Gobius fluviatilis.
17										"Crocodile"
18							Present			Chondrostoma nasus.
19										Leuciscus rutilus.
20										Tinea tinca.
21										Leuciscus erythrophthalmus.
22										Gasterosteus aculeatus.
23							Small.			Stizostedion lucioperca.
24										Gasterosteus aculeatus.
25										Scomber scombrus.
26	20 to 200.	×	Hair-like, nearly always localized.	Very numerous.	f					Aleyonella fungosa.
I					Membrane not subpersistent.	Many; number inconsistent.				
27		×			Membrane not subpersistent.	Many.	None			Callionymus lyra.
28					×	Many.	Pin-head to pea.	Spherical or irregular.	White.	Gast. aculeatus, Pygostens pungitius, Aphyia alba.
II					Membrane subpersistent.	Many; number inconsistent.				
29					Sporophorous vesicle 15 to 18 $\mu$ in diameter.	Many.	None			Cottus scorpio.
III					Membrane subpersistent.	8; number constant.				Decapoda.
30						8	None			Astacus fluviatilis.



## Tabular key—Continued.

Seat.	Pathologic effect.	Remarks	Genus.	Species.	Species No.
-----	-----	-----	Genus incert....	sp. incert.....	14
Air bladder.....	-----	-----	do.....	sp. incert.....	15
Body cavity.....	-----	-----	do.....	sp. incert.....	16
Mucosa and muscularis of intestine.	-----	-----	do.....	sp. incert.....	17
Roots of tongue.....	-----	-----	do.....	sp. incert.....	18
Heart; heart blood.....	-----	-----	do.....	sp. incert.....	19
Scales.....	-----	-----	do.....	sp. incert.....	20
Subsquamous.....	-----	-----	do.....	sp. incert.....	21
-----	-----	Fish collected near Kiel.	do.....	sp. incert.....	22
Branchiæ.....	-----	-----	do.....	sp. incert.....	23
Branchial "copules" See p. 187.....	-----	-----	do.....	sp. incert.....	24
-----	-----	-----	do.....	sp. incert.....	25
Body cavity.....	Death of polyzoon colony.	Capsules not yet demonstrated.	"Myxosporidium."	bryozoides.....	26
-----	-----	-----	Glugea.....	-----	I
Intra-fibrillar.....	Degeneration of muscle fiber.	-----	do.....	destruens.....	27
Subcutaneous.....	-----	-----	do.....	anomala.....	28
Subcutaneous.	-----	-----	-----	-----	-----
-----	-----	-----	Pleistophora.....	-----	II
Inter-fibrillar.....	No degeneration.	Diseased mass forming white streaks 5 or 6 by 3 mm.	do.....	typicalis.....	29
-----	-----	-----	-----	-----	-----
Striated muscles.....	-----	-----	Thelohania.....	-----	III
-----	-----	-----	-----	-----	-----
Striated muscles.....	Crayfish epidemic?	-----	do.....	contejeani.....	30

## Tabular key—Continued.

Species No.	Genera.	Spore.										
		Capsules.				Shell.		Vacuole.		Symmetry.		
		1 only.		2 or more in—		Bivalve.	Inclination of valve-junction plane to longitudinal.	Aniodinophile.	Iodinophile.	Antero-posterior.	Bilateral.	Supero-inferior.
		Obscure; spore minute.	Conspicuous; spore relatively large.	1 group (at anterior end).	2 separated groups. At each (anterior and posterior) end. In each (right and left) wing.							
31	Thelohania .....	×								0		
32	do .....	×						×		0		
33	do .....											
IV	Myxobolus .....		×	×		×	0°		×	0	×	×
34	do .....		×			×			×	0	Slightly imperfect.	very generally
35	do .....		×							0	×	
36	do .....			2 unequal.						0	Slightly imperfect.	
37	do .....			2						0	×	
38	do .....			(2)						0	×	
39	do .....			(2)						0	×	
40	do .....			(2)						0	×	
41	do .....			(2)						0	×	(×)
42	do .....			2					×	0	×	
43	do .....											
44	do .....									0	×	
45	do .....											
46	do .....			2		×	0°		×	0	×	×
47	do .....											
48	do .....			(2)						0	×	

## Tabular key—Continued.

Spore.											Index.			Presence of cornua of sporoplasm recognized.	Nuclei <i>ad plurimum</i> .	Species No.
Tail.				Outline on vertical view.	Dimensions.											
Absent.	Present.				Length in $\mu$ .	Breadth in $\mu$ .	Thickness in $\mu$ .	Length of capsules in $\mu$ .	Presence of filament; length in $\mu$ .	Capsular.	Caudal.	Ridge, presence and index.				
	Undivided.	Single.	More or less bifurcate.													
0				Pyriform	3 to 4				Extruded by ether only.						31	
0					5 to 6				15 to 20, extruded only by HCl and HNO <sub>3</sub> .						32	
				Pyriform											33	
× 0	×	×	×												IV	
×				Ovate											34	
×				Lanceolate-ovate.	16 to 18	7 or 8.									35	
×					11	7									36	
×				Lanceolate-ovate.						Very small					37	
0 (sometimes × 1)															38	
					7										39	
0 (sometimes × 1)				Oval or circular.											40	
															41	
				Flattened-ovoid.	10 to 12	8		6		Ab't 0.50				3	42	
					18 (error?)	12 (error?)									43	
						8	6 to 7.								44	
				Lenticular-oval.											45	
															46	
×				Oval	In size = sp. 61.										47	
×										Ab't 0.50					48	



## Tabular key—Continued.

Seat.	Pathologic effect.	Remarks.	Genus.	Species.	Species No.
Inter-fibrillar.	Muscular paresis.		Thelohania	octospora	31
Inter-fibrillar.			do	giardi	32
Muscles			do	macrocystis	33
			Myxobolus		IV
			do	unicapsulatus	34
Branchiæ; spleen.			do	piriformis	35
Kidney.			do	inequalis	36
Spleen; kidney.			do	brachycystis	37
Branchiæ(see p213).			do?	sp. incert	38
Cornea.					
Branchial lamellæ.			do?	mugilis	39
Branchial lamellæ.			do	sp. incert	40
Branchiæ			do	sp. incert	41
Fins and branchiæ.			do	oviformis	42
Branchiæ.			do?	cf. oviformis	43
Branchiæ.		Dimensions an error? (see p. 215).	do	sp. incert	44
Branchiæ			do	sp. incert	45
Branchiæ			do	mülleri	46
Branchiæ and fins.					
Branchiæ kidney.					
Ovary.					
Branchial arches			do	sp. incert	47
Branchiæ			do	bicostatus	48

## Tabular key—Continued.

Spore.													
Species No.	Genera.	Capsules.				Shell.		Vacuole.		Symmetry.			
		Obscure; spore minute. Conspicuous; spore relatively large.	1 only.		2 or more in—		Bivalve.	Inclination of valve-junction plane to longitudinal.	Amiodinophile.	Iodinophile.	Antero-posterior.	Bilateral.	Supero-inferior.
			1 group (at anterior end).	2 separated groups.									
				At each (anterior and posterior) end.	In each (right and left) wing.								
49	Myxobolus			2 <sup>1</sup> (see re-marks)			×	0°		×	0	×	×
50	do			2							0	×	
51	do			2			×	0°			0	×	
52	do			(2)									
53	do			(2)							0	×	
54	do			2			×	0°		?	0	×	×
55	do			2			×	0°		×	0	×	×
56	do			2			×	0°		×	0	×	×
57	do			(2)							0	×	
58	do			(2)						×	0	×	
59	do			(2)									
60	do			(2)			×						
61	do			2						?	0	×	
62	do			2			×				0	×	×
63	do			2			×			×	0	×	×
64	do			2						×	0	×	
65	do						×						
66	do						×						
67	do												
68	do			2 (nature??).							0	×	
	do												
	do												
	do												
69	do			2			×				0	×	×
70	do			2							0	×	?

## Tabular key—Continued.

Spore.												Index.			Presence of cornua of sporoplasm recognized.	Nuclei <i>ad plurimum</i> .	Species No.
Tail.				Outline on vertical view.	Dimensions.												
Absent.	Present.				Length in $\mu$ .	Breadth in $\mu$ .	Thickness in $\mu$ .	Length of capsules in $\mu$ .	Presence of filament; length in $\mu$ .	Capsular.	Caudal.	Ridge; presence and index.					
	Undivided.	Single.	Double from base.														
×				Flattened-ellipsoid.	12 to 15	9 to 11		4		Ab't 0.33				4	49		
×				Resembling sp. 92. Lenticular or oval.	11	7									50		
×					12	10	6		×						51		
×															52		
×															53		
×				Spatular.	14 to 17	8.5	5 to 6.	4 or 5.	×	0.30		About 0.33.		? (see p. 235).	54		
×				Broadly elliptic.	13.9	11	8		×	×		×		4	55		
×				Broadly elliptic.	14	10	5			A little less than 0.50.		0.25			56		
×				Broadly round-elliptic.	12										57		
×															58		
				Spherical.	9									3	59		
×				Almost exactly round.											60		
(see p. 240).				Subcircular. Transversely elliptic.	8	6 or 7.	5			About 0.60.		0.33		4	61		
×					6 or 7	8	5		×	About .50.				2	62		
×					Less than breadth.										63		
															64		
															65		
															66		
															67		
			2 (nature??)	Oval wider in front.						2 (nature??).					68		
×	×	×	×	Fusiform.				5.1 to 5.9.		Less than in sp. 80.		×	47.9		69		
0	×	0	0		15	5 to 6.				Hardly 0.50					70		

## Tabular key—Continued.

Species No.	Myxosporidium.						Cyst.			Host.	
	Size in $\mu$ .	Ectoplasm and endoplasm differentiated.	Pseudopodia.	Nuclei.	Pansporoblast.	Pansporoblast producing spores <i>ad plurimum</i> .	Vacuole.	Size in mm.	Shape.		Color.
49	.....	.....	Large and obtuse.	Many.	×	Usually 2.	.....	Present or absent .....			<i>Tinca tinca</i> .....
50	.....	.....			.....		.....	.....	.....	.....	.....
51	.....	.....	.....	.....	×	.....	.....	Present.....			<i>Barbus barbus</i> .....
52	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Leuciscus erythrophthalmus</i> .....
53	.....	.....	.....	.....	.....	.....	.....	Pin's head.	.....	Yellowish white.	<i>Phoxinus phoxinus</i> .....
54	.....	.....	.....	.....	.....	.....	.....	<i>Ad max.</i> 1.	Round or elliptic.		White.
55	.....	.....	.....	.....	.....	.....	.....	None (?).....			<i>Cyprinodon variegatus</i> .....
56	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Carassius carassius</i> .....
57	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Alburnus alburnus</i> .....
58	.....	.....	.....	.....	.....	.....	.....	Present.....			<i>Leuciscus rutilus</i> ..
59	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"Gardon" .....
60	.....	.....	.....	.....	.....	.....	.....	0.25 to 0.33.	.....	.....	<i>Coregonus fera</i> .....
61	.....	.....	.....	.....	.....	.....	.....	1.09 to 2.18.	Flat pustules.	White.	<i>Stizostedion lucio-perca</i> .....
62	.....	.....	.....	.....	.....	.....	.....	<i>Ad max.</i> 0.50.			.....
63	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Phoxinus funduloides</i> .....
64	.....	0	.....	.....	No pansporoblast membrane.	.....	.....	.....	.....	.....	<i>Merlucius merlucius</i> .....
65	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Gobio gobio</i> .....
66	.....	.....	.....	.....	.....	.....	.....	Present.....			<i>Perca fluviatilis</i> .....
67	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Leuciscus rutilus</i> .....
68	.....	.....	.....	.....	.....	.....	.....	Pea to large nut.	Oval..	White.	<i>Coregonus fera</i> .....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Tinca tinca</i> .....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Lucius lucius</i> .....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Stizostedion lucio-perca</i> .....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Leuciscus erythrophthalmus</i> .....
69	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Lucius lucius</i> .....
70	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Gasterosteus aculeatus</i> .....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	<i>Pygosteuspangitius</i> .....



## Tabular key—Continued.

Seat.	Pathologic effect.	Remarks.	Genus.	Species.	Species No.
Branchiæ, air bladder, liver, spleen, intestine, gall bladder (see p. 224.)			Myxobolus	ellipsoides	49
			do ?	sp. incert	50
Muscles (see also pp. 227-228). Splenic artery	Barbel epidemic.		do	sp. incert	51
			do	sp. incert	52
Surface of head			do	sp. incert	53
Surface of head			do	oblongus	54
Sides of body		Diseased mass fungoid, 4 by 2 to 10 by 4 mm.	do	lintoni	55
Body cavity			do	sp. incert	56
			do ?	obesus	57
Inner surface of opercle, pseudo-branchiæ.			do	cycloides	58
			do	sp. incert	59
Branchial mucosa			do	spheralis	60
Opercle, branchiæ, surface of head, fins.			do	sp. incert	61
Branchial lamellæ			do	globosus	62
Subsquamous			do	transovalis	63
Gall bladder		Each myxosporidium produces only 2 spores.	do ?	merlucii	64
Kidney, body cavity			do ?	sp. incert	65
Skin, scales			do ?	sp. incert	66
Muscles of sides of body			do ?	sp. incert	67
Subcutaneous and superficial inter-muscular tissue.			do ?	zschokkei	68
Cornea			do	see sp. 33	
Branchiæ			do	see sp. 41	
Opercle; branchiæ			do	see sp. 61	
			do	see sp. 52	
Ovary			do	cf. creplini	69
Renal tubules and ovary.			do	brevis	70
do					



## Tabular key—Continued.

Spore.														Index.	Presence of cornua of sporoplasm recognized.	Nuclei <i>ad pharium</i> .	Species No.
Tail.				Outline on vertical view.	Dimensions.												
Absent.	Present.				Length in $\mu$ .	Breadth in $\mu$ .	Thickness in $\mu$ .	Length of capsules in $\mu$ .	Presence of filament; length in $\mu$ .								
	Undivided.	Single.	Double from base.														
		More or less bifurcate.								Capsular.	Caudal.	Ridge; presence and index.					
0	×			Fusiform	20 to 30									71			
0	×			Ventricose-elliptic.	17.3	5.8					1 or a little more.			72			
0	×	0	0		Body 9	5.4						×		73			
0	×	0	0	Body lenticular or obovate.							2 to 3			74			
0	(rarely).	0	0	Body round-elliptic.	Body 10 to 11.	6 to 8.	4			0.50	3 to 4 or less.	×	×	75			
0	usually.	Many bifurcate-tipped.												76			
0	×	×	×	Lanceolate.	Body 19	5 to 6.	3		×	0.40	2			77			
0	×	×	Occasionally.	Body very narrow.	Of body, 3 to 4 times breadth.									78			
0	×	×	×	Body oval	Body 12	6	About 3.				3 to 4	×		79			
0 (very rarely)	×	×	×		Total 30 to 40.	4	6 to 8.				1	×		80			
0	×	×	×	Round or oval, sharp anteriorly.					×			×		81			
0	0	0	×	Body lenticular.	Body 8 to 10.									82			
0	0	0	×											83			
×				Widely transversely extended.										V			
					5	40								84			
						60								85			
					5 to 8.	65								86			

## Tabular key—Continued.

Species No.	Myxosporidium.						Cyst.			Host.
	Size in $\mu$ .	Ectoplasm and endoplasm differentiated.	Pseudopodia.	Nuclei.	Pansporoblast.	Pansporoblast producing spores <i>ad plurimum</i> .	Size in mm.	Shape.	Color.	
71	.....	.....	.....	.....	.....	(1)	.....	.....	.....	Gasterosteus aculeatus.
72	.....	.....	.....	.....	.....	.....	.....	.....	.....	Pygosteus pungitius.
73	.....	.....	.....	.....	.....	.....	.....	.....	.....	Acerina cernua.
74	.....	.....	.....	.....	.....	.....	Length about 2-18. Large.	Lenticular.	White.	Synodontis schal. ....
75	.....	.....	.....	.....	.....	.....	Pin's head.	Roundish.	White.	Aphredoderus sayanus.
76	.....	.....	.....	.....	.....	.....	1	.....	.....	Hybognathus nuchalis.
77	.....	.....	.....	.....	.....	.....	1	Subspherical.	White.	Coregonus fera .....
78	.....	.....	.....	.....	.....	.....	Present	.....	.....	Ameiurus melas. ....
79	.....	.....	.....	.....	.....	.....	0.44 to 1.09.	.....	Whitish.	Rhamdia sebæ.....
80	.....	.....	.....	.....	.....	.....	.....	.....	.....	Pseudoplatystoma fasciatum.
81	.....	.....	.....	.....	.....	.....	10 to 30 by 7 to 20.	Spherical or oval.	Yellowish white.	Lucius lucius.....
82	.....	.....	.....	.....	×	1 (??)	.....	.....	.....	Perca fluviatilis ....
83	.....	.....	.....	.....	.....	.....	Filbert to small walnut.	.....	.....	Coregonus fera .....
V	.....	.....	.....	.....	.....	.....	None	.....	.....	Lota lota.....
84	Form variable, maximum diameter 35 or 40 $\mu$ ; endoplasm destitute of spherules; pseudopodia ectoplasmic, lobed, filiform variety absent; myxosporidium destitute of prolongations.						None	.....	.....	Onus tricirratum ....
85	Maximum length 85, maximum breadth 20 $\mu$ ; form variable, usually subfusiform; anterior end round, posterior attenuate, round, or multilobate; pseudopodia ectoplasmic <i>ad plur.</i> 8, subfiliform, limited to anterior end, maximum length $\frac{1}{2}$ that of myxosporidium; movements rapid.						None	.....	.....	Dasyatis pastinica..
86	Form irregular, variable; prolongations 1 to 5 with endoplasmic axis and ectoplasmic covering, immovable, maximum length twice that of central portion of myxosporidium; pseudopodia ectoplasmic, lobed; place of origin variable.						None	.....	.....	Lophius piscatorius.

## Tabular key—Continued.

Seat.	Pathologic effect.	Remarks.	Genus.	Species.	Species No.
Renal tubules and ovary.			Myxobelus	medius	71
do.			do	creplini	72
Surface of head			do	strongylurus	73
Subcutaneous inter-muscular tissue.			do	monurus	74
Surface of head			do	macrurus	75
Branchial arches			do	sp. incert	76
Base of second dorsal fin.			do	cf. linearis	77
Membrane lining branchial cavity.			do	linearis	78
Branchial lamellæ.			do	schizurus	79
Orbit			do		
Branchiæ			do	psorospermicus	80
Branchiæ.			do	kolesnikovi	81
Interstitial muscular connective tissue.			do		
Muscles			do	sp. incert	82
Kidney			do	diplurus	83
Gall bladder		Bisporogenesis a generic feature.	Ceratomyxa		V
Gall bladder			do	arcuata	84
Gall bladder		Myxosporidium bisporogenetic.	do	agilis	85
Gall bladder		Myxosporidium bisporogenetic.	do	appendiculata	86

## Tabular key—Continued.

Species No.	Genera.	Spore.												
		Capsules.					Shell.		Vacuole.		Symmetry.			
		1 only.		2 or more in—			Bivalve.	Inclination of valve-junction plane to longitudinal.	Anidionophile.	Iodinophile.	Antero-posterior.	Bilateral.	Supero-inferior.	
		Obscure; spore minute.	Conspicuous; spore relatively large.	1 group (at anterior end).	2 separated groups.									
					At each (anterior and posterior) end.	In each (right and left) wing.								
87	Ceratomyxa			2			×	90°		0	0	×	(sporoplasm unilateral).	
VI	Chloromyxum			2			×		0	0	0	×		
88	(Sphaerospora). do			2			×			0	0	×		
89	do			2			×	90°		0	0	×		×
90	do			(2)			×	90°			0	×		
91	do			(2)			×	Apparently 90°.			0	×		
92	do			2						0	0	×		
VI	Chloromyxum			4			×	90°(?)		0	0	×		
93	sens. strict. do			4							0	×		
94	do			4						0	0	×		
95	do			4			×	90°(?)		0	0	×		
96	do			4							0	×		
VII	Cystodiscus				×		×	90°			×	×		×
97				1 at each end.			×	(valves oblique to transverse plane.)	90°		×	×		×

## Tabular key—Continued.

Spore.													Index.	Presence of cornua of sporoplasm recognized.	Nuclei <i>ad plurimum</i> .	Species No.	
Tail.				Outline on vertical view.	Dimensions.												
Absent.	Present.				Length in $\mu$ .	Breadth in $\mu$ .	Thickness in $\mu$ .	Length of capsules in $\mu$ .	Presence of filament; length in $\mu$ .	Capsular.	Caudal.	Ridge, presence and index.					
	Undivided.	Single.															Double from base.
		More or less bifurcate.															
×	-----	-----	-----	Transversely subisocetes-triangular.	8 to 12	100	-----	-----	×	-----	-----	×	-----	-----	87		
×	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	VI		
×	-----	-----	-----	Subspherical.	8 to 12	-----	-----	-----	-----	-----	-----	×	-----	-----	88		
×	-----	-----	-----	Transversely elliptic.	6	8	3 to 3.5.	×	About 0.50.	-----	-----	Very small.	-----	-----	89		
×	-----	-----	-----	-----	Less than breadth.	-----	-----	-----	-----	-----	-----	-----	-----	-----	90		
×	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	91		
×	-----	-----	-----	Oval, pointed anteriorly.	10 to 12	7	-----	-----	-----	-----	-----	-----	-----	-----	92		
×	-----	-----	-----	Subspherical.	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	VI		
×	-----	-----	-----	Cuneate-ovate.	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	93		
×	-----	-----	-----	Cuneate-ovate.	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	94		
×	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----		
×	-----	-----	-----	Nearly spherical.	5 to 7	-----	-----	-----	-----	-----	-----	×	-----	-----	95		
×	-----	-----	-----	Subspherical, mucronate anteriorly.	<i>ad max.</i> 8.	-----	-----	-----	-----	-----	-----	-----	-----	-----	96		
×	-----	-----	-----	Fusiform	12 to 14	9 to 10	-----	-----	-----	-----	-----	(X)	-----	-----	VII		
×	-----	-----	-----	Round-fusiform.	-----	-----	-----	4 to 5 times length of spore	-----	-----	-----	×	-----	-----	97		

## Tabular key—Continued.

Species No.	Myxosporidium.						Cyst.			Host.	
	Size in $\mu$ .	Ectoplasm and endoplasm differentiated.	Pseudopodia.	Nuclei.	Pansporoblast.	Pansporoblast producing spores <i>ad plurimum</i> .	Vacuole.	Size in mm.	Shape.		Color.
87	Spherical or oval; young stages amœboid, colorless, older yellowish; pseudopodia lobed, motile; endoplasm riddled with spherules, 3 to 4 $\mu$ in diameter containing pigment granules.							None			Galeorhinus galeus Galeus mustelus.
VI											
88								None			Gasterosteus aculeatus. Pygosteus punctatus. Phoxinus phoxinus.
89								None			Bufo lentiginosus...
90											Acerina cernua
91						1(?)					Lota lota
92	1250 to 1500.	Apparently membraneless.									Leuciscus rutilus, Leuciscus erythrophthalmus.
VI								None			
93	29 to 88.				1 to 4.	1		None			Raja batis
94	29 to 147.	×	×	Many.	×	1		None			Galeus mustelus... Scylliorhinus canicula. Scylliorhinus stellaris. Pristiurus melanostomus. Squalus acanthias... Squatina squatina... Torpedo torpedo... Torpedo marmorata. Raja clavata... Dasyatis sp. Cephaloetherus aquila.
95		×	Ectoplasmic, lobed.					None			Leuciscus cephalus.
96	<i>ad max.</i> 75.	Membraneless.			Probably.	2 (??)		None			Lota lota
VII											
97	<i>ad max.</i> 1500 to 2000.	×					Numerous "vesicles".	None			Bufo agui Cystignathus ocellatus.



## Tabular key—Continued.

Seat.	Pathologic effect.	Remarks.	Genus.	Species.	Spec. No.
Free in gall bladder. ....		Myxosporidium bi-sporogenetic.	Ceratomyxa .....	sphaerulosa .....	87
Free in gall bladder. ....			Chloromyxum .....		VI
			(Sphaerospora).		
Renal tubules and ovary. ....			do .....	elegans .....	88
Renal tubules and ovary. ....					
"Accidentally" in kidney. ....			do .....	ohlmacheri .....	89
Renal tubules; urine and surface of bladder. ....	Pressure effects.		do .....	perlatum .....	90
			do .....		
Ovary .....			do .....	sp. incert .....	91
Pseudobranchiae .....			do .....	dajardini .....	92
Branchial lamellae. ....					
Free in gall bladder. ....			Chloromyxum .....		VI
			(sens. strict.)		
Free in gall bladder. ....		Posterior border of spore radiate-toothed.	do .....	incisum .....	93
Gall bladder. ....			do .....	leydigii .....	94
Gall bladder. ....					
Gall bladder. ....					
Gall bladder. ....					
Gall bladder. ....					
Gall bladder. ....					
Gall bladder. ....					
Gall bladder. ....			do .....	fluvialito .....	95
Free in urinary bladder. ....			do .....	muconatum .....	96
Gall bladder. ....		No "nucleus" seen	Cystodiscus .....		VII
Gall bladder. ....			do .....	immersus .....	97

## Tabular key—Concluded.

Species No.	Genera.	Spore.											
		Capsules.					Shell.		Vacuole.		Symmetry.		
		1 only.	2 or more in—				Bivalve.	Inclination of valve-junction plane to longitudinal.	Aniodinophile.	Iodinophile.	Antero-posterior.	Bilateral.	Supero-inferior.
		Obscure; spore minute. Conspicuous; spore relatively large.	1 group (at anterior end).	2 separated groups.									
				At each (anterior and posterior) end.	In each (right and left) wing.								
98	Cystodiscus			2 at each end.		× (valves perpendicular to transverse plane)	90°			×	×	×	
VIII	Sphæromyxa			2 groups (position?).		×			0	?	?	?	
	do			1 at each "extremity" (end??).		×			0				
IX	Myxidium				×	0			0	0	×		
100	do				1 or 2 in each wing.	0			0	0	×		
101	do				2, in 2 groups (position?).	×			0	But 1 plane of symmetry, viz; the valve-junction plane.			
102	do				1 in each wing.					?	×		





## Tabular key—Concluded.

Seat.	Pathologic effect.	Remarks.	Genus.	Species.	Species No.
Body cavity .....			Cystodiscus??	diploxys .....	98
			Sphæromyxa .....		VIII
Gall bladder .....			do .....	balbianii .....	99
Gall bladder.					
Excretory tract (see p. 107).			Myxidium .....		IX
Urinary bladder .....			do .....	lieberkühni .....	100
Gall bladder .....			do?? .....	incurvatum .....	101
Gall bladder.					
Gall bladder.					
Gall bladder.					
Bile ducts .....			do? .....	sp. incert. ....	102

## NON-MYXOSPORIDIAN.

1. *Psorospermia sciænæ-umbræ* Robin, 1853.<sup>1</sup> Pl. 1, figs. 1-4.

Hist. Nat. des Végét. Parasites, pp. 314-321, pl. 14, figs. 14, 15; pl. 15.

Robin defined the species as follows:

Cellulæ ovoideæ vel raro sphericæ aut ovoideo-elongatæ; coriaceæ, intus granulose, achromaticæ, luteo-succineæ vel luteo-fuscæ. Long., mm. 0.027; lat., mm. 0.018; sphericæ, mm. 0.017. In stratis (coloniæ) indefinitis, vel cylindricis, filamentosis, circulatim flexuosis, continuis coherentes, raro isolatæ.

*Hab.* Infra membranam mucosam cavi branchialis insitam in septo abdomino-branchio sciænæ-umbræ.

The species consists of three varieties. The description is Robin's condensed and rearranged.

## VARIETY 1.—(Robin's plate 15, figs. 2a, b; 4a, b; 6.)

*Microscopic.*—Cells ovoid (27 by 18  $\mu$ ) or spherical (diameter 17  $\mu$ ), a little flattened on one side, having an amber-yellow tint with a white shining reflex, strongly refringent, resembling fat drops; ovoid cells a little flattened with clearly defined borders and double contoured walls (1  $\mu$  thick) rupturable by pressure, cell-contents then escaping. Contents clear, yellow, homogeneous, strongly refracting, liquid, in which float 5 to 8 or more, strongly refringent granules, 1  $\mu$  in diameter. Cells not altered by acetic acid or ammonia.

*Macroscopic.*—Cells cohering into grayish yellow, flexuous cylinders (colonies) 0.5 mm. in diameter (plate 15, fig. 1); length sometimes 1 m. or more. Cylinders convoluted, *circular, endless*, usually united in pairs by a double or triple delicate transparent connective tissue sheath (fig. 2e, f, g), the whole forming a delicate string rolled upon itself, in every direction (pl. 1, fig. 1a of this paper) into a flattened spherical, lobulated or nonlobulated mass, whose size varies from that of a nutlet to that of a fist.

## VARIETY 2.—(Robin's plate 15, figs. 2c, d; 4c, d.)

*Microscopic.*—Cells ovoid, white, colorless, transparent, with a shining reflex, with more numerous and larger granulations than the other varieties.

*Macroscopic.*—Cells united into opaque, milk-white, filamentous, continuous, endless cylinders, either by simple cohesion or by amorphous matter, which latter forms around each cylinder a (hardly perceptible) thin enveloping membrane (plate 14, figs. 2c, d; 4c, d). These filaments are only visible under a lens, being only  $\frac{1}{10}$  to  $\frac{1}{8}$  as thick as the cylinders of the first variety.

<sup>1</sup>This species was first described as a constituent part of the body of the host by Robin, in his paper "Anatomie d'un organe découvert sur l'ombre (*Sciæna umbræ*) read to the *Société philomatique* Nov. 28, 1846 (Procès verb. d. la Soc. philomat. Paris, 1846, p. 140; also Journ. l'Institut No. 683, Feb. 3, 1847, Paris, xv, p. 41). Not seen; *fide* Robin, 1853, p. 314.

VARIETY 3.—(Robin's plate 15, figs. 3; 5a, b; 8.)

*Microscopic*.—Cells regularly or irregularly ovoid, a little smaller than those of the first variety, brownish yellow, presenting a peculiarity found in no animal cell, viz, a round opercle.<sup>1</sup> Cells unaffected by acetic and nitric acids, and by ammonia.

*Macroscopic*.—Colonies of variety 3, consisting of small lenticular, or irregular brown or white masses scattered here and there at the base of or below the lobes, and especially over the submucous surface of the parasitic convoluted-string mass.

(1) Brownish masses.—2 to 4 mm. thick, composed of masses or colonies of irregular, cupped, operculate cells, the whole enveloped by a layer of cellular tissue containing very fine capillaries. Masses sometimes sufficiently numerous to color quite an area of the mucosa blackish brown. Further, when the convoluted-string mass is absent, brown bodies may occur in the same situation. These bodies are ordinarily accompanied by small pea-sized, whitish corpuscles, composed of round granules measuring about 0.20 mm., formed of strongly united fibers of cellular tissue wound around a small transparent, apparently calcareous, body. It contains in the center 1 to 8 or 12 cells, furnished with an opercle similar to that above described.

(2) Whitish masses.—Composed of grains formed of 2, 3, 4, or 12 (rarely 1) cells, surrounded by a thick cellular tissue layer, the fibers of which are strongly united by amorphous finely granular matter, the whole forming rather hard, white, spherical or ovoid grains,  $\frac{1}{8}$  to  $\frac{1}{4}$  mm. in size, often clearer in the center.

Calcareous granules forming an oval or circular mass (fig. 5) with sharply defined borders (the latter sometimes split); granules forming whitish, more or less flattened, friable, irregularly lobulated, pea-sized miliary masses. Granular mass destitute of vascularity, the vessels being confined to the tissue sheath.

Some masses are hard, yellowish white, of variable form, composed of operculate cells, calcareous granules, and a great number of very large, quadrilateral or rhomboidal, tabular crystals, the latter often piled up, insoluble in acetic acid, in which only the calcareous granules disengage some bullæ of gas. Calcareous granules also occur without crystals, being in this case whiter and less yellowish.

*The convoluted string (cordon enroulé)*.—As described above, the cells of varieties 1 and 2 form continuous (endless) cylindrical filaments, those of variety 1 forming *yellow* filaments, those of variety 2 forming *white* filaments. The convoluted string is usually<sup>2</sup> formed of 6 of these

<sup>1</sup> Robin gives the size of the opercle as 0.06 mm., but as he says the cells are smaller than those of the first variety (whose length is 0.027 mm.) this must be an error, possibly for 0.006 mm.

<sup>2</sup> Sometimes, however, only 2 filaments (instead of 6) are present, viz, 1 large yellow filament (instead of 2), and 1 (not 4) thin white filament. Also (very rarely) the convoluted string contains only 1 (instead of 6) white filament (variety 2) and 2 or 3 successive enveloping sheaths.

filaments (arranged in two series, *a* and *b* below) together with a connective tissue sheath (*c* below).

(*a*) First series, composed of one yellow filament (variety 1) and two white filaments (variety 2), the latter applied one along each side of the yellow filament. One of the white cylinders is always flexuous, the other always straight and without undulations.

(*b*) Second series, consisting, like the first, of a yellow filament (variety 1) accompanied by two semitransparent, hyaline, whitish filaments, which resemble the previously described filaments in being continuous and endless, *but which appear not to be composed of cells*. They consist only of a thin wall filled with a semiliquid, finely granular substance. One of these whitish filaments is flexuous and undulating; the other, instead of being straight throughout its whole length, undulates a little from place to place.

(*c*) Sheaths formed of connective tissue of the host, penetrated by delicate capillaries.

*Parasitic mass* (as a whole).—Showing through the thin covering of transparent mucous membrane of branchial cavity as a grayish or whitish mass of convoluted strings (varieties 1 and 2), strewn with small brown masses (variety 3) of the size of a pea. Size of parasitic mass varying from that of a millet seed to that of a large goose egg. Sometimes voluminous on one side and small on the other; sometimes composed of two or three separate lobes. Form inconstant, generally consisting of round or elongated lobes. Arteries and veins few, extremely delicate; derived from vessels of neighboring muscles, which, with the loose submucous tissue, form the only bond between the mass and the tissues of the host. Injection with mercury (of the connective tissue sheath, described above under variety 1) demonstrates that the mass consists of closed lobules. When filled with mercury, no escape of the metal occurs unless greater pressure produces rupture. When very small, the mass may be unrolled and shown to consist of a convoluted string.

*Habitat, etc.*—Submucous connective tissue of branchio-abdominal septum (between scapular and last branchial arch) of *Scienia umbra*. Among 9 fish (male and female) examined in September, it was absent in 4. The size of the 5 hosts varied from 1.30 m. to 1.70 m. Sometimes, but rarely, variety 3 exists alone, the usual condition, however, being that varieties 1 and 2 are present together and are accompanied by small colonies of variety 3.

*Nature.*—Robin regards it as referable to the Diatoms. Lieberkühn<sup>1</sup> says that:

The psorosperms of some marine fishes recently described by Robin behave in every respect like Trematode eggs.

Whatever other view be taken of its affinities, this species is certainly not myxosporidian. As remarked above (p. 72), the generic name must follow the type species.

<sup>1</sup> Müller's Archiv., 1854, pp. 10-11.



**2. *Lithocystis schneideri* Giard, 1876.** Pl. 2, figs. 1, 2.

Sur une nouvelle espèce de psorospermie (*Lithocystis schneideri*) parasite de l'*Echinocardium cordatum*; Compt. Rend. Acad. Sci. Paris, 1876, LXXXII, pp. 1208-1210; transl. Ann. Mag. Nat. Hist., London, 1876, XVIII, pp. 192-194; also see Bütschli, Bronn's Thier-Reich, I, pp. 590, 602; figured in Schneider's Tablettes Zoologiques (*vide* Pfeiffer, Die Protozoen als Krankheitsserreger, p. 49); *ib.* Perrier, 1893, Traité de Zool., p. 459.

**Cyst unknown.**

*Plasmodium*.—Forming shining black (pigmented) irregular masses. Size varying from that of a point to 10 mm. by 4 or 5 mm., aspect and consistence similar to that of the myxomycete plasmodia; surface of mass showing hyaline cysts with a structureless membrane, 2 mm. or less in diameter, containing one or more, rarely several, white points (crystal masses) and spores, the latter arranged in an irregular sphere. Spores situated at the extremities of filaments, which radiate from a central point, at which is a nucleus of a yellowish substance. Each spore is sustained by 2 filaments tangential to the extremities of its shorter axis. Wherever possible (principally in the larger cysts), the spores become, at maturity, so rearranged as to form a number of little groups; spores cohering by their previous peripherally-placed portions.<sup>1</sup> At the same time the two filaments become applied to each other so as to form a single tail-like filament 3 or 4 times the length of the spore. The little groups then resemble colonies of *Flagellata*, but the tail-like filament remains motionless. The coherence of the spores is due to a secretion produced at the adhering ends of the spores.

Crystals insoluble in acetic acid, soluble in nitric acid, broken up at maturity of cyst, forming a sort of network, which seems to function somewhat similarly to the capillitium of the Myxomycetes in the dissemination of the spores. Pigment of plasmodium believed to be derived from host. The amœbæ present in the fluid of the body cavity of the host are regarded as originating from the falciform corpuscles, which are seen to slowly lose their form, and Giard believes them to produce by their union and growth the plasmodia.

*Spores*.—Fusiform, length 6 to 10  $\mu$ , breadth 1 to 2  $\mu$ . Some cysts (apparently the smaller) produce microspores, others megaspores, both of which classes differ from the ordinary variety of spore mainly in being more inflated towards the middle. Spore with 2 filaments (subsequently becoming 1, as above described) tangential to the shorter axis. Contents of spores merely a granular protoplasm, or from 3 to 6 falciform corpuscles in course of formation, arranged around a central residual mass, which latter is finally reduced to 2 or 3 strongly refringent granules, and may disappear at maturity.

*Effects*.—The parasite causes the formation of small nodosities on the inner surface of the test, which may enable us to recognize the presence of this parasite in fossil *Echinodermata*.

<sup>1</sup>I. e., the portion corresponding to the "anterior pole" of a myxosporidian spore.

*Habitat*.—Body cavity of *Echinocardium cordatum* (sea-urchin), particularly against the test between the mouth and subanal plastron, and especially toward the conical point which terminates the plastron inferiorly; also frequently on the inner side of the actinal curvature of the intestine.

*Nature*.—Giard says:

I have found nothing resembling the Gregarines, and the whole of the facts observed lead me to approximate the parasite not to the lower animals, but to the lower plants (*Myxomycetes* and *Chytridinea*); on the other hand, the spores being identical with those described as arising in the cysts of the Gregarines, one may ask whether the relation of the *Psorospermia* to the Gregarines is not a relation of parasitism rather than of genetic bonds.

Prof. Bütschli, the only other author who has (as far as I know) commented upon this form, says:<sup>1</sup>

It may indeed be possible that an organism as yet unfortunately only briefly described by Giard, his so-called *Lithocystis schneideri*, occupies a sort of middle ground between Gregarines and *Myxosporidia*, since it combines the plasmodioid nature with the production of spores similar to the *Myxosporidia*, together with the development of sickle-shaped germs in these spores. Unfortunately, however, as said, *Lithocystis* has not yet been fully described, so that the decision is at present somewhat difficult.

Prof. Lankester<sup>2</sup> places *Lithocystis* among the genera of the *Myxosporidia*. Pfeiffer<sup>3</sup> says that this species forms "a transition to a still unknown side."

*Remarks*.—First as to Giard's opinion, which is entitled to especial weight as being derived directly from a study of the form itself, while Bütschli's is here to a certain extent an opinion of an opinion. In Giard's article I fail to find the slightest indication of a desire to approximate *Lithocystis* to the *Myxosporidia*. True he calls it a "psorosperm," but he uses this term in a very vague sense, its scope appearing to be at least equivalent to that of the term *Sporozoa*. Further he states that:

The whole of the facts observed lead me to approximate the parasite not to the lower animals but to the lower plants (*Myxomycetes* and *Chytridinea*).

Then he argues that since the spores of *Lithocystis* are identical with the spore-like contents of the gregarine cysts, perhaps the latter (which he also denominates "psorosperms") are not gregarine spores, but gregarine parasites.

Prof. Bütschli, however, says that while its spores agree with those of the Gregarines in containing falciform germs, *Lithocystis* possesses in common with the *Myxosporidia*, a plasmodioid nature and the production of similar spores.

<sup>1</sup> Es wäre sogar möglich, dass ein bis jetzt leider nur flüchtig von Giard beschriebener Organismus, seine sogenannte *Lithocystis schneideri*, eine Art Mittelstufe zwischen Gregariniden und Myxosporidien einnimmt, da er das plasmodienartige Wesen mit Erzeugung ähnlicher Sporen wie die Myxosporidien, sowie der Hervorbildung sichelförmiger Keime in diesen Sporen vereinigt. Leider ist jedoch, wie gesagt, die *Lithocystis* noch nicht eingehend beschrieben so dass ihre Beurtheilung bis jetzt etwas schwer fällt (Bronn's Thier-Reich, 1882, I, p. 602).

<sup>2</sup> Encycl. Britan., 1885, 9 ed., XIX, p. 855.

<sup>3</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., p. 49.

However much (or little) this may prove as to the stability of body-form in the Gregarines, I can not see that it proves anything as regards the *Myxosporidia*. Further, I can not see any resemblance between the spores of *Lithocystis*, which contains falciform germs and no capsules, and the capsulate myxosporidian spores.

Perrier includes it among the *Myxosporidia*.

Finally, the following excellent paper (seen and incorporated at the last moment) seems to settle the question beyond doubt, and serves to remove almost the last "transition" form from the taxonomic doubtful list:

L. Cuénot: Commensaux et parasites des Échinodermes; Rev. Biolog. Nord France, Lille, v, Oct. 1, 1892; *Lithocystis schneideri* Giard, pp. 4-6, plate 1, figs. 1, 2.

The following is an abstract:

*L. schneideri* is a perfectly typical monocystid Gregarine; the gregarine stage probably occurs in the digestive tube, being rarely encountered in the body cavity, the Gregarine probably encysting soon after traversing the intestinal walls. In fact, cysts are encountered upon, but not attached to, the intestinal wall. In the body cavity the Gregarine was always found (whether accidentally or otherwise) in the midst of a mass of cysts. Gregarine ovoid, about  $65\ \mu$  long, protoplasm very vacuolate, inclosing a rather large number of clinorhombic crystals, which also occur in the cysts; a voluminous nucleus, with large nucleoli, is present.

Masses of the spherical cysts, well described by Giard, occur of all dimensions (*ad max.* 1 to 2 mm.) in different regions of the body, especially on the intestine and on the oral surface. They inclose a considerable number of spores and a voluminous rest of segmentation riddled with the same crystals that occur in the Gregarine.

Spores of variable dimensions (megaspores  $24\ \mu$ , microspores  $12\ \mu$ ), ovoid, distal end neatly truncate, proximal end rounded; spores limited by a unique refringent integument (endospore) situated at the extremities of small, very delicately walled tubes, which latter form a sort of more or less undulating episore.

Spores arranged, at least in the large cysts, in a number of small, radial groups, formed by the convergence of the tubes to a common center. Contents of young spores granular; of mature spores 8 falciform corpuscles (4 at each end), and a central rest of segmentation. The falciform corpuscles are probably expelled on the death of the host, and other Echinocardiums naturally become infected by swallowing the sand containing them.

Pigment identical with the products of dissimulation spread through the tissues of the host; if specially condensed around the cysts, it is as a result of the [increased tissue] expenditure necessitated by their considerable growth.

The presence of small nodosities on the test could not be determined.

The cysts, united into more or less voluminous masses, are surrounded by a considerable mass of black pigment and of amœboid cells, the latter very evidently *Echinocardium* amœbocytes accumulated around the foreign bodies. The latent life of the cysts is probably not very long, as there are frequently seen, apparently in process of degeneration, small ones inclosing only empty spores absolutely devoid of nuclei.

As in all the other Monocystids studied, the *Lithocystis* spore has dissimilar poles, the one truncate, the other rounded and furnished with a long tube. The structure of the cysts is appreciably different from all other known Monocystids.

## 3. Genus et sp. incert. Pl. 2, fig. 3.

Parasite of *Gadus callarias*, Müller & Retzius, 1842, Ueber parasitische Bildungen; 1. Ueber eine eigenthümliche Krankheit der Schwimmblase beim Dorsch, *Gadus callarias*, Müller's Archiv., pp. 193-8, pl. 8, fig. 1; *ib.*, Rayer, 1843, Rayer's Archiv. de Méd. comp., I, pp. 284, 287-9, pl. 9, fig. 14; *ib.*, Leydig, 1851, Müller's Archiv., p. 22, mention only; psorosperms of *G. callarias*, Robin, 1853, Hist. Nat. Végét. Parasites, pp. 291, 309, pl. 14, fig. 1; ? psorosperm of bladder of codfish, St. George, 1879, Ueber die Feinde der Fische, Circ. 3, Deutsch. Fisch-Verein, p. 178, and Rep. U. S. Fish Com. for 1878 (1880), VI, p. 510; Myxosporidian? Coccidian? Bütschli, 1882, Bronn's Thier-Reich, I, p. 591, footnote; psorosperm of *Gadus merluccius* (error)<sup>1</sup> Balbiani, 1883, Journ. de Microgr., VII, pp. 145, 280; *ib.* (error),<sup>1</sup> Balbiani, 1884, Leçons sur les Sporozoaires, p. 122; ? psorosperms of cod, v. d. Borne, 1886, Handb. d. Fischzucht u. Fischerei, p. 211.<sup>2</sup>

Adult unknown.

*Cyst*.—Unknown. Pathologic formation consisting of a whitish-yellow, pasty mass drawing out into threads of a greasy, dirty character, mostly diffuent (evidently less advanced), with a firmer portion surrounding the softer, in quantity about 6 fluid ounces, odorless even after several days exposure to the air; microscopic examination showing it to consist of the below-described corpuscles with a small amount of granular matter, the whole imbedded in and held together by a mucoid substance.

*Spore*.—Best described by comparison to a ribless ventricose *Navicula* or to Agardh's *Frustula coffeaformis*, elliptic, length pretty uniformly 14 to 17  $\mu$ , consisting of two valves, the substance of which is shown by complete decomposition upon ignition to be nonsiliceous; their carbon incinerates with difficulty; each valve of an elliptic outline with a convex outer and a concave inner surface, usually in contact with its fellow of the opposite side by the inwardly convex middle portion of its border, the borders of the valves diverging towards their ends; sometimes obliquely set so as to be in contact by one end only, sometimes in contact for their whole length, thus forming a lenticular corpuscle, along the median line of which the junction can be plainly traced; middle of valves cemented together by a mass occupying part of the body cavity; mass showing more or less plainly a number of large and small granules, and apparently destitute of a surrounding membrane.

*Development*.—By far the largest number of the corpuscles are destitute of a surrounding membrane; some were, however, observed heaped

<sup>1</sup> Prof. Balbiani misquotes the name of the host as "the merluche, *Gadus merluccius*." The context (he refers to the diseased air bladder) renders it evident that this is an error for *G. callarias*, and not (as might be expected) for *G. merlangus*. Inferentially from his language he regards the form as myxosporidian. Perugia (Boll. Scientif., Pavia, 1890, XII, p. 134) has followed Balbiani's misquotation.

<sup>2</sup> "With the cod [*Gadus morrhua*] and mackerel [*Scomber scombrus*] the development of large psorosperm-lumps with great emaciation and later ulceration is very well known, and not rarely there occurs in freshwater fishes, from the same cause, a great mortality."

3 or 4 together into irregular clumps. Many such clumps had no surrounding membrane, but some showed such a membrane containing several corpuscles. The features of the latter bodies were plainly discernible through the enveloping membrane. The corpuscles at this stage are unsplit, the valves being united for their whole length, forming a lenticular corpuscle. Further, similar cysts were seen which showed no developed corpuscles, but only large granules. Finally, a number of separated valves may be seen. From these facts Müller concludes that the corpuscles in question develop several in a cyst, are set free unsplit, subsequently the valves separate, at first partially, at last probably entirely, and then perhaps the cycle is repeated.

*Habitat*.—Air bladder of *Gadus morrhua* (= *callarias*), cod.

*Nature*.—Robin includes it among the "psorosperms."

Dr. L. Wittmack<sup>1</sup> refers to this as a "psorosperm."

Concerning this form Prof. Bütschli<sup>2</sup> says:

It appears to me quite questionable whether these psorospermiform corpuscles of the air bladder of *Gadus callarias* are to be referred to the *Myxosporidia* proper or to the *Coccidia*. Their structure appears to approximate itself rather to the latter; especially in the absence of the polar capsules so characteristic of the *Myxosporidia*.

I can see no myxosporidian structure in it, and have, therefore, omitted it from the subclass.

*Effects*.—Mucous membrane of the air bladder red and swollen, infiltrated by the parasitic mass. Tail unusually thin and shrunken, the soft parts being markedly atrophied, the muscular tissue having disappeared. Further observation must determine the constancy and causality of relation between the two conditions. Such atrophy is apparently not rare in *Gadus*, as the fishermen at Bohuslän knew the disease and informed Müller that it rendered the fish unfit for food.

Müller says that the difference between this form and the psorosperms of fresh-water fishes is as great as that between different genera of animals.

#### *Atrophy of tail of Merlangus merlangus.*<sup>3</sup>

The following observation probably can not be better placed than as an appendix to the similar disease of *G. morrhua* just described. Among the Mediterranean fishes collected by Mr. Peters, Müller and Retzius noted a *Gadus merlangus* affected with complete atrophy of the tail muscles, the tail being composed of nothing but skin and bone—not the slightest trace of muscular tissue remaining. The junction of the normal and atrophied tissue was abrupt and was situated at the root of the tail. Unfortunately, the air bladder had not been preserved.

<sup>1</sup> Beiträge zur Fischerei-Statistik d. deutsch. Reichs, 1875, p. 191, footnote.

<sup>2</sup> Bronn's Thier-Reich, 1882, I, p. 591, footnote.

<sup>3</sup> Müller and Retzius, 1842, Müller's Archiv., p. 198; see also p. 172.

## 4. Genus et sp. incert. Pl. 4, fig. 1.

Entozoan of *Salmo fario*, Valentin, Ueber ein Entozoon im Blute von *Salmo fario*, Müller's Archiv., 1841, pp. 435, 436, pl. 15, fig. 16; *ib.* Leydig, 1851, Müller's Archiv., pp. 11, 12; cf. Davaine, Traité des Entozoaires, Paris, 1860, p. III.

*Amœboid stage*.—In blood obtained by puncture of the abdominal aorta of *Salmo fario* (brown trout) Valentin found, besides the blood corpuscles, some dark globules similar to round pigment cells. They have a quick, tremulous motion, also a definitely locomotive one. Observed for some time, a clear "tail" comes into view, which later elongates; there thus becomes revealed an elongate animal with a rapid motion, mostly of rotation, effected by 1 to 3 variable processes of one side of the body. Anterior and posterior parts clear; middle portion containing numerous dark corpuscles, perhaps pigment particles which it had eaten. When rolled up into a ball it often had the appearance as though each club-shaped process of the body contained one of the globules (pl. 4, fig. 1e). No finer structure could be detected. Size 7.5 to 12.5  $\mu$ . Sometimes a round opening appeared to be present at the anterior end. The posterior end is somewhat striate. The variable processes always appear in the drawing as they would be seen in the microscope on the right side. Perhaps the club-shaped peduncles are to be reckoned as such. In drawn blood they remain living from 6 to 8 hours.

*Nature*.—These bodies are, Valentin says, probably referable to *Proteus* or to *Amœba*, of which they certainly form a new species, different from all of Ehrenberg's. Doubting at first whether these organisms really belonged to the blood, Valentin investigated the whole fish. He failed to find, either on the peritoneum, or in the kidneys, intestines, air bladder, brain, etc., any trace of these infusorial Entozoa. Only in the fourth ventricle (the favorite seat of the microscopic intestinal worms) did he find a single specimen. On the contrary, they were so numerous in the blood that often a single droplet contained 10 or more. The blood itself presented nothing worthy of note. The fishes examined showed numerous examples of *Ascaris obtuso-caudata* Zedér. No other intestinal worms were found.

Leuckart<sup>1</sup> says:

Still less is the gregarine nature of the entozoan found by Valentin in the blood of the trout to be mistaken.

Lieberkühn regarded it as an amœba. It could not, he says, be a Gregarine, as it lacks a nucleus.<sup>2</sup>

Although this form has been referred to the *Myxosporidia* by Leydig, the evidence to sustain such reference is wanting, and at present its myxosporidian affinities can not be regarded as proven.

<sup>1</sup> Archiv. f. physiol. Heilkde, 1852, XI, p. 431.

<sup>2</sup> Müller's Archiv., 1854, pp. 11, 12. For Lieberkühn's subsequent change of view as to the necessity of the presence of a nucleus in the Gregarines, see pp. 95, 96.

**5. *Balbiana rileyi* Stiles, 1893. Pl. 3, figs. 1-5.**

(Psorosperms of mallard duck, Leidy, 1875, Proc. Acad. Nat. Sci. Phila., xxvii, p. 125).

*Balbiana rileyi*, Bull. 3, Bur. An. Ind., Dept. Agric., pp. 80-84, pl. 2, figs. 1-5.

Dr. Leidy's description may be summarized as follows:

Cyst, oval, white, 2 to 4 mm. long, 0.7 mm. thick. Contents, myriads of fusiform corpuscles. Spores fusiform corpuscles resembling minute navicellæ; length  $17\mu$ ; habitat, encysted in interstices of muscles of the mallard duck (*Anas boschas* L.).

*Nature*.—Leidy says that—

Similar bodies were first discovered by the late Prof. Müller and described by him under the name of psorosperms. They have been repeatedly observed since by Retzius, Robin, and others, in the muscles and other parts of fishes, and they are usually regarded as vegetable parasites. Though the mallard is not a fish-eater, the bird may have become infected by eating infected fish.

From this extract it might not unnaturally be supposed that in this instance "psorosperm" referred to a myxosporidian.

Recently Dr. C. W. Stiles has reëxamined the subject. He studied material from two hosts and five localities, including one lot labeled:

Oval, smooth bodies, no limbs. In muscles of Mallard. *Anas boschas*. Dr. E. Coues. Ex. Jan. 29, 1890.

The following is the diagnosis:

Parasite 1 to 6 mm. long by 0.48 mm. broad; rather fusiform, ends not sharply pointed. Cuticle not striated, about  $2\mu$  thick. Central core not coloring and not containing falciform bodies. Peripheral zone as broad as central core (0.16 mm. to 0.16 mm.) or even broader, coloring in various liquids (acid carmine; methyl blue), containing numerous falciform bodies. Form of meshes irregular but elongated radially. Falciform bodies 12 to  $14\mu$  long, more pointed at one extremity than at the other; containing a very distinct nucleus ( $2\mu$ ) which stains clearly in acid carmine or methyl blue, and which contains several chromatophile granules; vacuole quite indistinct.

*Habitat*.—Intermuscular connective tissue of ducks, the shoveler or shovelbill duck or spoonbill duck (*Spatula clypeata*), and the mallard or tame duck (*Anas boschas*). Development unknown.

North America. (?) Philadelphia, Pa. (Coues; Leidy); St. Louis, Mo. (Riley); Clear Lake, Cal. (Brett); Minnesota (Lüger); Quebec (Bélanger).

Type material deposited in the U. S. National Museum, in the Bureau of Animal Industry, and in collection of Stiles, Washington, D. C. Specimens are also to be found in the Army Medical Museum, Washington, D. C., and in collection of Leidy, University of Pennsylvania, Philadelphia, Pa.

In conclusion, although "measly duck" is not very appetizing in appearance, there are no grounds for believing that it is dangerous to man.

## 6. Genus et sp. incert. Pl. 4, figs. 2-8; pl. 5, figs. 1-11.

Pilzsporen of *Cyclops*, Claus, 1863, Die freilebenden Copepoden, Leipzig, p. 87; *Myxosporidia*? of *Cyclops*, of *Diapt. caruleus* and of *Diapt. richardi*, Schmeil, Beiträge z. Kenntn. d. freilebenden Copepoden Deutschlands, Ztschr. f. Naturwiss. Halle, 1891, LXIV, pp. 19-21; Entoparasitische Schläuche der Cyclopiden Schewiakoff, Ueber einige ekto-, and entoparasitische Protozoen der Cyclopiden, Bull. Soc. Imp. Nat. Moscow, 1893, pp. 2, 15-26, pl. 1, figs. 17-34.

## Claus says:

The bodies formerly<sup>1</sup> designated by me "spores of fungi," with which I have many times found the body-cavity of *Cyclops* entirely filled, I have unfortunately not been able to observe again in later times. From the earlier period, sufficient notes on these bodies unfortunately are lacking, so that I am compelled to leave undetermined their nature and their relation to *Parhistophyton ovatum*, so full of significance through the disease of the silk-worm.

To his quotation of part of the above Schmeil (p. 21, footnote 1) adds:

"The organisms observed by me are, however, certainly not spores of fungi" [italics his own].

## Schmeil further says (abstract):

I have observed another parasite in nearly all the *Cyclops* of the Halle [Page 19] region, further in the specimens seen of *Diapt. caruleus* Fisch. and *D. richardi* Schmeil.

As this parasite is relatively very frequent—though absolutely (*ständig*) [Page 20] rare—one soon learns to tell the affected animals with the naked eye by their striking gray color. Their movements are unaffected. Microscopic examination shows individual parts of the body strikingly dark (in *Cyclopids* and *D. richardi* Schm., black; *D. caruleus* Fisch., dark brown); often the whole thorax, the abdomen, and even the tail, the first antennæ, and natatory feet are either entirely or partly filled by this dark mass. On closer examination this dark color is seen to be due to an innumerable host of small fusiform or crescentic corpuscles, whose form (plainly perceived by pressure-rupture of the copepod shell) places them as psorosperm-like bodies. From Schmeil's description and drawings, Bütschli considered them *Myxosporidia*. Size very variable; besides very small corpuscles, one meets with larger ones 3 or 4 times the smallest, but the sizes of all those occurring in the same individual are always nearly equal. These corpuscles appear to possess a firm membrane, immediately within which a clear zone is situated. No differentiation of contents could be observed. Water and glycerin do not alter the form.

Origin of these corpuscles unknown; repeated attempts to infect [Page 21] healthy animals failed. Multiplication by division seems proven by the occurrence of two or several corpuscles lying close together, often in contact lengthwise; often, however, with their blunt poles surrounded by a common membrane. Therefore, in case the explanation generally given is correct, a double division in the transverse and longitudinal axes appears to take place.

On account of the lack of infected animals it is exceedingly difficult to reach safe conclusions concerning these conditions.

Such was the state of the subject when Schewiakoff began his investigations. The following are his results:

This condition has been observed at all seasons, first on *Cyclops strenuus* Fisch. taken from under the ice of a pool (clay ditch near Schlettau).

<sup>1</sup>Place not stated; or whether published.



Tubes rather frequent in very many fresh-water copepods, the affected individuals being distinguishable at first glance from the healthy by their opacity, the places where the parasites lie appearing dark. If in great number, the *Cyclops* appear completely opaque, and, indeed, according to Schmeil (loc. cit., p. 20), may appear dark brown to black. Discoloration caused by larger or smaller tubes filled with pyriform, spore-like corpuscles; tubes occurring in body-cavity, and various other places, as the thorax, abdomen, tail, natatory feet, and first antennæ; sometimes in so great numbers that no part of the body is free from them. Spores in some places not in tubes but free in body-cavity, then always found directly on the muscles.

These parasites were probably those which Claus observed in copepods and regarded as spores of fungi; also extremely probably those noted by other observers, in various crustacea, e. g., Henneguy in *Palæmon rectirostris* and *P. serratus*, Henneguy and Thélohan in *Crangon vulgaris* and *Astacus fluviatilis*, and Garbini in *Palæmonetes varians*. However, it cannot with certainty be asserted that the parasites found in the last-mentioned crustaceans are identical with the *Cyclops* parasite, as to the short communications no figures<sup>1</sup> are added, and the authors in question were unable to follow the whole developmental history.

*Technique.*—The affected *Cyclops* was isolated in a drop of water on the slide and covered with a cover glass provided with wax feet, fixed in position by careful pressure on the angles of the cover-glass, so that it remains quiet and can be conveniently observed even with a high power (apochr. 4 mm.). Between the observations the *Cyclops* was at first kept in a hanging drop in the moist chamber, but lived only a few (2-3) days, dying partly from starvation, partly from other unfavorable conditions. Consequently the *Cyclops* was next kept in a watch-glass of water, thus securing necessary food supply. Thus kept, it lived 14 days, allowing the development of the parasites to be followed. Several individuals were kept simultaneously and examined 2 to 4 times a day. Investigation of dead or crushed specimens is not to be recommended, as great bacterial development soon disturbs the study. For observation of the finer anatomical features and the developmental stages, the parasites were isolated by crushing the host and observed with very high powers (homog. immers. apochr. 2 mm., oc. 12 and 18). For fixation, picro-sulphuric, and chromo-aceto-osmic acids; for stains, alum carmine, hæmatoxylin; also methyl violet, safranin, and fuchsin. Examinations were made partly in water, partly in glycerin.

1. *Amœbiform stage.*—Met with in all parts of the body; most easily observed on the first antennæ. Form amœboid-variable, globular or elongate; dimensions varying from 7 $\mu$  long by 3 $\mu$  broad, to 20 $\mu$  long by 6 $\mu$  broad. Plasma finely granular, capable of emitting on all sides blunt, lobulate, hyaline pseudopodia, always possessing a nucleus (pl. 4, fig. 2 N) and a small contractile vacuole (c. v.). Nucleus globular, showing the familiar vesicular structure, that is, in its interior, a globular, homogeneous, more strongly refringent and more deeply staining nucleolus [*Binnenkörper*]. Contractile vacuole constantly situated near the border, in the end of the body which during progression is hindermost, pulsating about once every 30 seconds; no food vacuole perceptible.

This amœba ordinarily creeps about over the epithelial and muscle cells and probably feeds upon the same, as, although not directly observed, many epithelial cells were seen destroyed, and upon them amœbæ.

After attaining a certain size the amœbæ gradually cease their movements, draw in their pseudopodia, and encyst themselves.

The amœbæ may fuse to large plasmodies; several such fusions of 2 or 3 amœbæ (pl. 4, fig. 8) were directly observed. Size of plasmodies varying with size and

<sup>1</sup>The author is partly in error as regards the absence of figures. They will be found in the papers of Henneguy and Garbini.

number of constituent amœbæ from  $18\mu$  long by  $8\mu$  broad to  $48\mu$  long by  $23\mu$  broad. In fusing the amœbæ adhere closely to one another, finally after some time fusing into one mass, which can then undergo further movements. Nuclei (pl. 4, fig. 8 N) of plasmode vesicular, 2 to 3 according to the number of constituent amœbæ. Union or fusion of the nuclei not directly observed; regarded, however, as very probable, as frequently pretty large plasmodes of  $22\mu$  and  $18\mu$  (doubtless [Page 19] formed by fusion of 2 or 3 amœbæ) were seen containing only 1 large, vesicular nucleus (pl. 5, fig. 2 N). Besides, plasmodes seen to originate by fusion of 3 amœbæ and to contain nuclei, showed on the next day only 1 large nucleus.

Contractile vacuole not demonstrable with certainty in fusion plasmodes; its presence, however, not regarded as impossible; the plasma, on the contrary, contains so many vacuoles as to appear vacuolate or frothy. Motion of plasmodes rather slow. Plasma in the next 24 hours undergoing a change; the frothy, vacuolate structure changing to a finely granular condition, the vacuoles vanishing. Nucleus, also, no longer visible; probably transformed by division into several globular strongly refringent bodies (pl. 5, fig. 3 N), though this was not directly observed. Motion of plasmode in this stage quite slow, ceasing entirely after some time; encystment following in 1 or 2 days.

2. *Encystment*.—The encystment of simple small amœbæ and the alterations in their body plasma is first described; afterward the process with the fusion plasmodes. With the small amœbæ encystment begins when they have attained a certain size. They gradually draw in their lobulate pseudopodia and acquire an irregular, more or less oval or pyriform shape. Locomotion still takes place, though very slowly, small ragged pseudopodia being still emitted. After about 1 hour this movement also ceases and the amœba revolves slowly, gradually rounding itself off and assuming with a state of rest a nearly globular form. After about 10 hours it has transformed itself into a proper cyst (pl. 4, fig. 3) about  $10\mu$  in diameter, [Page 20] consisting of a plainly bordered, extremely thin membrane and finely granular contents, in which individual, small, strongly refringent granules, a vesicular nucleus (N), and a contractile vacuole (*c. v.*), which now pulsates markedly more slowly, are perceptible.

After about 24 hours (pl. 4, fig. 4) the membrane appears markedly thicker, double contoured, and the strongly refringent granules have increased in number. The nucleus no longer appears vesicular, but homogeneous and rather strongly refringent. Contractile vacuole still always visible, although now pulsating extremely slowly (about once in 5 minutes).

After another 24 hours (pl. 4, fig. 5) the protoplasm appears strongly brilliant, the contractile vacuole has vanished, and the nucleus is not perceptible. In their places are observed several round, strongly refringent structures (probably proceeding from division of the nucleus), differentiated from the other cyst-plasma granules already mentioned, by their more considerable size and their affinity for stains. Though the falling to pieces of the nucleus was not directly observed, the granules may with tolerable safety be admitted to have originated through nuclear division. Schewiakoff thinks that first the nucleus divides, and about 10 hours later the spores (pl. 4, fig. 6) are formed, since around every nucleus a portion of the protoplasm delimits itself from the remainder.

Encystment of plasmodes occurs in the same way. Locomotion becomes continually slower until finally it is extinguished. The plasmode then rounds itself off, acquires a somewhat elongate oval form, which, as also the size, varies greatly. It then secretes a thin membrane, which envelops it closely on every side (pl. 5, fig. 4).

[Page 21] In 1 to 2 days the membrane becomes markedly thicker, then appearing homogeneous, strongly refringent and double contoured. During the next day spore formation begins.

Plasmode encystment thus differs from that of simple amœbæ only in the fact that the conditions observed in the amœba cyst (granular state of the protoplasm, vanishing of the nucleus, or, in other words, its peculiar falling to pieces into individual small nuclei) wear themselves off with the plasmodes during their motile stage.

3. *Spore formation*.—Beginning about 3 days after encystment; not originating through successive division of the nucleus and protoplasm, the nucleus falling to pieces into several small, strongly refringent corpuscles (pl. 4, fig. 5 N), around which, later, portions of protoplasm segregate themselves from the remainder. In this way the spores are formed. Thus in a simple amœba cyst, 10 hours after the falling to pieces of the nucleus, 6 spores (pl. 4, fig. 6) were seen, each with a small globular nucleus. Besides these, the cyst still contained plasma in which were seen, along with many small, strongly refringent granules, isolated small, round nucleiform structures (N). About 24 hours later the number of spores had doubled; nevertheless, there was still present undifferentiated plasma as well as nuclei. After 24 hours more the number of spores had so increased as to entirely fill the cyst; no free protoplasm remained (pl. 4, fig. 7).

Spore formation in the plasmode cysts (also accurately followed) takes place in the same way. In plasmode cysts containing numerous small nuclei (very probably originating through successive divisions of the nucleus) are formed small bodies, globular to oval, delimited from the surrounding protoplasm by a delicate membrane (pl. 5, fig. 4), fine-grained, some allowing a small, globular nucleus to [Page 22] show through. After about 6 hours these bodies acquire a somewhat pyriform shape, the membrane becomes thicker and sharper, the protoplasm more hyaline, the nucleus thus becoming more distinctly visible. This transformation proceeds so that after 24 to 36 hours the bodies are pyriform, sharply contoured, completely hyaline spores (pl. 5, fig. 5), in which a globular nucleus is always plainly visible. Along with this transformation new spores are formed from the surrounding protoplasm, until all the free protoplasm is used up, the cysts transforming themselves into spore cysts or spore tubes. Number of spores in cyst variable, dependent upon the size of the cyst, whose diameter varies from about 10 $\mu$  (simple amœba cysts) to 30 to 60 $\mu$  (plasmode cysts); often also elongate-oval spore tubes are found 70 $\mu$  long and 24 $\mu$  broad.

Spores: Length, 3.3 to 4 $\mu$ , oval or pyriform (pl. 5, fig. 8), rather strongly refringent, completely hyaline, bounded exteriorly by an extremely thin homogeneous layer, the *pellicula*. In the broader end of the body a globular, very strongly refringent, homogeneous nucleus (N), 1.6 $\mu$ , is found. The spores thus originating still further increase through a somewhat oblique-running, transverse division, the nucleus dividing karyokinetically (pl. 5, fig. 10a-l). Division was followed *intra vitam*, and the study completed in specimens fixed with chromo-aceto-osmic acid and stained with hæmatoxylin. Nuclear division, requires about  $\frac{1}{2}$  hour, and proceeds in about the same way as that of the micronucleus of the ciliated Infusoria. The membrane or external border-layer of the nucleus remains quiescent during the whole process, only in the last stages (pl. 5, fig. 10h) appearing some- [Page 23] what indistinct preliminary to reappearing with distinctness in the daughter nuclei.

Owing to the small size of the nucleus, karyokinesis could be followed only in the principal steps. The first alteration observed in the nucleus is a marked increase in size; simultaneously it loses its homogeneous character, acquiring a netted, honeycomb-like structure (pl. 5, fig. 10a) with tolerably strongly staining granules. This netted form passes into an elongate, striate-fibred structure (b), the nucleus at the same time enlarging and assuming an ellipsoid form whose long axis coincides with that of the spore. Between the nuclear poles run meridional striæ, in which the chromatin granules are imbedded. These latter become concentrated toward the equator, when a so-called nuclear plate (c) forms, which consists of baculiform

chromosomes which lie close to the delicate but perceptible threads of the achromatic spindle. Regarding the spore from the posterior end (*d*), the chromosomes are seen to be 8, and to lie rather peripherally. After the formation of the nuclear plate, a halving of the chromosomes takes place in the equator (*e*), the halves receding until they reach the poles of the nucleus (*f*). Meanwhile the spore has changed from pyriform to ellipsoidal, and the hyaline protoplasm has become by degrees granular.

As soon as the chromosomes have reached the poles an annular constriction becomes visible at the equator of the spore as well as of the nucleus (*g*); between the daughter chromosomes, achromatic spindle fibers are very plainly observed. Soon at the equatorial constriction, an annular thickening of the spore membrane forms (*h*), running obliquely to the longitudinal axis, from above downward. In this stage the membrane (or external border) of the nucleus becomes indistinct and the fibers of the achromatic spindle also do not stand out so sharply. The annular constriction grows gradually inward and subsequently forms the partition wall dividing the 2 spore halves. Meanwhile the familiar after-formation of the chromosomes (*i*) takes place in the daughter nuclei, the nuclear membrane becomes again more distinct, and the achromatic fibers are scarcely visible.

[Page 24] In the next stage (*k*) a distinct division wall between the 2 spore-halves is observed and the daughter nuclei show a finely reticular appearance, whence result later homogeneous nuclei (*l*). Division of the daughter spores soon takes place.

A somewhat peculiar phenomenon was often observed. Among the many dividing spores some were encountered with their anterior (narrower) ends more or less intimately united (pl. 5, fig. 11*a-b*). Schewiakoff could observe neither the union nor the division of the 2 spores. As, however, they differ essentially from the observed division stages, it may be questioned whether we have not here to do with a conjugation. This conjecture is strengthened by the presence, in the usually homogeneous nucleus, of structures (pl. 5, fig. 11*a*), which remind one of the nuclei of many conjugating Infusoria.

The spores increase considerably in number, the spore cyst becoming ultimately entirely filled by them. After a couple of days the cyst bursts at one place (pl. 5, fig. 6) and the spores are scattered with considerable force around the body cavity. They then mostly lie (pl. 5, fig. 7) in great masses, or in groups of 3-5, on the muscles.

As to the further fate of the spores nothing definite is known. After about 2 days they lose their homogeneous appearance and show an indication of a granular condition. Four days later they possess an irregular form (pl. 5, fig. 9) with finely granulated protoplasm and a distinct homogeneous nucleus. Size 3 to 4  $\mu$ . No movement or transition into the amœboid stage (which transition is, however, regarded as very possible) could be demonstrated. The manner of infection also remains unexplained.

*Nature.*—Without doubt Schewiakoff says, sporozoan. Schmeil, he says, considered it myxosporidian. (See above; the conjecture was Bütschli's.) These parasites, especially the spores, have a great similarity to those found by Henneguy and Thélohan in some decapods and by them ranked with the *Myxosporidia*.

Schewiakoff, however, doubts the myxosporidian nature of the *Cyclops* parasite. Henneguy and Thélohan gave their forms this place on account of their discovery of the filament. They only observed this extrusion a few times under the action of hydrochloric or nitric acid, and it was difficult to evoke. Since Schewiakoff could not discover either filament or capsule, he did not feel justified in referring the *Cyclops* parasite to the *Myxosporidia*. He, however, neglected to employ strong acids and alkalis, which is, he says, perhaps the reason of the failure.

It appears tolerably certain that the *Cyclops* parasite is not identical with their *Thelohania* species, as the latter have no amœboid stage, the globular cysts (sporoblasts of H. & Th.) are of constant size (14 $\mu$ ), and have always 8 spores with a different structure.

The presence of a contractile vacuole in the adult, the peculiarities in the process of spore formation, the falling to pieces of the nucleus, the apparent absence of pansporoblasts, the occurrence of reproduction only at and as the end of the life cycle, and the further multiplication by the division of fully formed spores, all absolutely contraindicate any myxosporidian affinities. Further, the constant presence of pigment<sup>1</sup> corroborates this conclusion, which is still further enforced by negative evidence from the structure of the spore, the most prominent feature of which is, of course, the absence of the capsule. Indeed it seems safe to go further and say that no organism with a contractile vacuole can, in the present state of our knowledge, be regarded as a sporozoan (cf. Lankester, *Encycl. Britan.*, 1885, 9 ed., XIX, p. 854).

PROBABLY MYXOSPORIDIA. (Imperfectly described.)

7. Genus et sp. incert.

Amœbiform corpuscles of gills of *Cyprinus brama*, Lieberkühn, 1854, Müller's *Archiv.*, pp. 6, 7; ? ib. of heart-blood of same fish,<sup>2</sup> p. 14; cf. also Müller, Müller's *Archiv.*, 1841, pp. 491-2.

*Cyst.*—Membrane so transparent that all details could be as well seen before as after expression of its contents. Contents "psorosperms" and amœbiform corpuscles, or amœbiform corpuscles only.

*Myxosporidium.*—Numerous, partly granular, partly granule-free, the latter usually smaller than the former, alterations of appearance very manifold, processes rather sharp than blunt, size not equal to that of a blood corpuscle of the fish; granules extremely small, held together by a mucoid substance.

*Spore.*—Unknown.

*Habitat.*—Encysted in the gills of *Abramis brama* L. (bream) in November.

*Remarks.*—Its habitat suggests that this species is probably a *Myxobolus*.

8. Genus et sp. incert.

Sarcode masses of *Perca fluviatilis*, Lieberkühn, 1854, Müller's *Archiv.*, p. 353.

*Cyst.*—Apparently no true cyst (see mention below of membrane).

*Myxosporidium.*—Consisting of granular protoplasm presenting a great similarity to that of *Chloromyxum mucronatum*, very variable in appearance, oval, lenticular or dendroidly branched. Size 27 to 440  $\mu$  ( $\frac{1}{80}$  to  $\frac{1}{6}$ '''); some specimens surrounded by a structureless membrane, others not; sometimes the whole substance is seen to have fallen apart

<sup>1</sup> While it is, of course, not contended that this alone would suffice to prove a species nonmyxosporidian, pigmentation, such as exists in the *Cyclops* cyst, would raise a strong presumption against its myxosporidian nature.

<sup>2</sup> Those [amœbiform corpuscles] of the heart blood of *Cyprinus brama* completely parallel in their form the above-described amœbiform masses found on the gills of the fish, and are differentiated among themselves in the same way as the gill forms [i. e., they are either granular or granule-free]. Their movements are, on account of their small size, difficult to observe.

into globules (pansporoblasts) every one of which contains 2 spores or perhaps only faint indications of such.

*Spore*.—Not described.

*Habitat*.—On branchiæ of *Perea fluviatilis* L. (yellow perch).

9. Genus et sp. incert. Pl. 6, fig. 1.

*Myxosporidium* of *Lota vulgaris*, Lieberkühn in Bütschli, 1882, Bronn's Thier-Reich, I, pl. 38, fig. 20.

No description.

*Habitat*.—Gall-bladder of *Lota lota* L. (= *vulgaris*), ling.

10. Genus et sp. incert. Pl. 6, fig. 2.

*Myxosporidium* of *Lota vulgaris* Lieberkühn in Bütschli, 1882, Bronn's Thier-Reich, I, pl. 38, fig. 24.

No description.

*Habitat*.—Branchiæ of *Lota lota* L. (= *vulgaris*), ling.

11. Genus incert. ("Myxosporidium") *congru* Perugia, 1891. Pl. 6, figs. 3-8.

*Myxosporidium congru* Perugia, Boll. Scientif., Pavia, XIII, pp. 24-5, figs. 15-20; *ib.*, Thélohan, 1892, Bull. Soc. philomat. Paris, IV, p. 166; *Chloromyxum* ?? *congru*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 419; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Myxosporidium*.—Found attached to a calculus-like compact mass consisting of fungus (probably *Penicillium*), bacteria, and crystals. Individuals numerous, form variable, movements incessant, slow, amœboid. Perugia observed in some a clear space which he believed to be a "vacuole" (pansporoblast), but careful examination failed to detect the spores.

*Habitat*.—Gall-bladder of *Leptocephalus conger* (= *Conger vulgaris*), eel, collected in August, 1890.

The generic name *Myxosporidium* is not in good standing (see p. 206). In the absence of knowledge of the spores the generic reference of this form is entirely uncertain.

12. Genus et sp. incert. Pl. 7, figs. 1-3.

Psorosperm of *Notropis megalops*, Linton, Bull. U. S. Fish Com. for 1889 (1891), IX, pp. 359-61, pl. 120, figs. 1-3; *ib.* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIII, p. 97.

*Cyst*.—Globular, discrete or aggregated into clusters, white, with minute patches of black pigment from host; size varying from 2.5 mm. (single cysts) to 7 by 5 mm. (clusters); wall composed of connective tissue, thin, collapsing when punctured, indistinguishable from deeper layers of derma, staining deeply with ammonia-carmin. Contents, a milky fluid.

*Myxosporidium* unknown.

*Spore*.—Somewhat top-shaped, one end broadly rounded, slightly flattened, the other tapering to a point, length 17  $\mu$ ; breadth 10  $\mu$ ; thickness 6  $\mu$ . Shell, thick and strong, resisting for a long time the action of sulphuric acid and of potassium hydrate solution; shape not changed by those reagents, by acetic acid or by glycerin, not staining with carmine; showing when viewed on edge an elevated ridge [junction of valves?]. Capsules could not be detected. Protoplasmic contents appear in most cases to be finely granular. Tail absent.

*Habitat*.—Subcutaneous tissue of all regions of the body of *Notropis megalops* Raf. (red-finned minnow) taken in Black River, Lorain County, Ohio, 6 miles above Lake Erie, September 1, 1890 (also October 5, 1891; see below). Collector, Mr. L. M. McCormick. Identification by Dr. D. S. Jordan.

With this species of fish were taken *Noturus miurus*, *Catostomus teres*, and *Moxostoma macrolepidotum*, and, in the immediate neighborhood, *Ictalurus* and *Roccus*. None of these, however, were affected.

*Effects*.—The epidermis of the fish is sometimes marked by dark purplish blotches. Scales are absent from the surface of the cyst in most cases, although a few were observed quite loosely attached to one of the larger clusters. All of the fishes appeared to be in fair condition.

Mr. McCormick has kindly furnished me the following additional information:

The fish were taken in the pool formed by Day's Dam, near the center of Sheffield Township, Lorain County, Ohio. Although he has diligently explored the streams of Lorain County for material for his "*Descriptive List of the Fishes of Lorain County, Ohio*,"<sup>1</sup> he has never seen *N. megalops* infested by this parasite except in this very limited locality. The same day that specimens were first secured there he seined Black River thoroughly from Elyria to below Day's Dam (distance 10 miles), but saw no other diseased specimens. In spite of the admitted fallibility of negative results, he believes this parasite to be restricted to a very narrow geographical range. Fish first taken September 1, 1890 (about a dozen); a few more October 5, 1891 (the first time of seining the pool that year).

### 13. Genus et sp. incert. Pl. 7, fig. 4.

Psorosperms of *Gasterosteus aculeatus*, Lieberkühn, 1854, Muller's Archiv., pp. 9-10, 22, 24, 354-7, pl. 2, fig. 28, pl. 14, figs. 9-12.

The following observations by Lieberkühn relate to a puzzling form found on *Gasterosteus aculeatus* (stickleback). His remarks are to me somewhat obscure, and I am not certain that I always understand his meaning. For that reason the translation is a literal one.

[Page 9] I am still in entire ignorance as to what becomes of the psorosperms of *Gasterosteus*. In the skin of this fish Gluge found cysts filled with entirely structureless granules which had a marked similarity to those of the Gregarines. Johannes Müller has confirmed this discovery. I investigated about 100 cyst-bearing specimens selected from a corresponding number of healthy sticklebacks. Among 10 fishes there was, in the spring, about 1 available; in late autumn, on the contrary, only 1 in about 100. The cysts varied greatly in size; the largest attract attention at once, the smaller are only to be discovered upon close examination. They have a very irregular form, mostly rod-shaped, and contain ordinarily the structureless granules mentioned by Gluge. A few contained bodies with more definite structure and characters, reminding one of the psorosperms, for which reason I will so name them. They are all nearly globular and somewhat smaller than the ordinary psorosperms; they consist of a transparent membrane, within which I have observed 3 kinds of contents, namely, in some a single small globule which is not large enough to come in contact with the membrane by its upper surface; in others

lay, between the surrounding membrane and the upper surface of this [Page 10] small globule, a small mass of exceedingly fine granules; in still others the globule appeared to have divided, as 3 or 4 smaller globules were present. Several of the smaller cysts contained a far more finely granular mass than

<sup>1</sup>Bull. 2, Oberlin College, Ohio.

that described by Gluge; I was not able to discover anything definite therein. So far I have found the largest cysts to contain only Gluge's structureless granules. In any case these facts are not yet sufficient to establish a developmental series.

In recapitulating and summarizing his results (the order of such summary and the place therein of the following extract showing that it refers to and is intended as the summary of the preceding quotation) Lieberkühn says:

In the skin of *Gasterosteus* occur, besides the grain-containing cysts discovered by Gluge, also such as contain psorosperms of peculiar species.

In a subsequent article Lieberkühn again discusses these problematical organisms. He says:

[Page 354] As regards the psorosperm-like bodies of the stickleback, to which I have already, in my preceding article, devoted some words, I have now succeeded in making the requisite observations preliminary to a knowledge of their developmental history. After I had, in the course of the preceding autumn and winter, examined in vain several thousand specimens of *Gasterosteus* for those cysts, I refound them first in March of this year in great numbers. Of the cysts discovered by Gluge I am not at present able to give any explanation, other than that they are entirely different from the ones now to be discussed.

[Page 355] The latter I have frequently found, to the number of 30 or more, distributed over the skin, the fins, and the cornea; some had bored through the fins and floated with both ends free in the water; others lay closely appressed to the skin for their whole length; others again were detached on one side. Individual fishes had their tail-ends so beset that scarcely anything of the scales could be seen. Their usual form is cylindrical; rarely they are ellipsoidal or spherical. They strike the eye with the first glance at the fish. The length of the rod-shaped is from  $\frac{1}{2}$  to 1 line; the greatest diameter of a cross-section about one-fifth line or more. The membrane of the cyst is plainly visible, and one can easily obtain it for examination by removing it by means of a knife. I could not discover any structure in it. The contents present great variations. In some I found nothing but an albuminous substance, in which fat-like granules were suspended in great numbers; these were globular and measured 0.001<sup>mm</sup>. If one moves them to and fro under the cover glass for some time many of them flow together to large oily drops. Other cysts contain partly these, partly much smaller but apparently similar granules. In still other cysts the granules of the smaller variety were united by a mucous substance into globules; many of these were distinguished by a much larger fatty granule lying in the middle between the smaller ones, and which often had an irregular form.

In still others this was seen to be 2 or 3 times as large, and in these cases the small granules were usually entirely absent; furthermore, the whole psorosperm had a proportionately greater size. The diameter of such a body was 0.008<sup>mm</sup>, of the nucleus [*Kern*] 0.005<sup>mm</sup>, of the fine granules about 0.0007<sup>mm</sup>. In the largest, granules began to appear anew, and it sometimes seemed as though they separated themselves from the nucleus. The expression nucleus has here no further significance than that which it receives through the investigation. Sometimes I was able to observe the same isolated, when for some unknown reason the surrounding membrane became ruptured and expressed its contents. It showed nothing but what one could see through the surrounding membrane. When the psorosperm dries on the cover glass it acquires an entirely different refrangibility, the sharp contour disappearing and not reappearing when water is added. In some cases I found also in fresh cysts such nuclei of feeble refrangibility within the smaller psorosperms. They vary greatly in size; were often simultaneously provided with granules, such being, however, often absent. In order to learn the further alterations of the cyst contents, I kept a number of cyst-bearing fish alive for some weeks in my room. Apparently the thin cysts increased in circumference, and then contained only the



largest kinds of psorosperms. Several fish lost their cyst contents entirely. In an apparently half-empty cyst microscopic investigation showed the following objects:

1. The largest form of the psorosperms, with a nucleus [*Kern*] of 0.005''' in diameter and containing many of the smallest granules.

2. The largest form of the psorosperms, with a much smaller "nucleus," namely, of 0.003''' in diameter, and filled with a much larger number of the smallest granules.

3. Corpuscles of the same size with the same striking "nucleus," with the same granules, but with a far less prominent surrounding membrane.

4. Corpuscles of the same kind, but without demonstrable membrane, slowly projecting a part of the body substance and again withdrawing it, whence resulted marked changes of form.

[Page 356] 5. Corpuscles with all these characters; also provided with such a "nucleus," but with a diameter twice as great.

In order to determine whether the structures described occur in the organism of fishes and migrate in the spring to the external skin for the purpose of reproduction, I examined a series of the individual parts of the fish. In

[Page 357] the blood I found moving colorless corpuscles, which agreed not with those of the fish, but much more closely with those destitute of grains and nuclei, originating from the psorosperms. And I also discovered in the kidneys of *Gasterosteus* receptacles with tailed psorosperms and the various developmental stages of the same, just as they occur in the gills of the pike. As the cysts often beset the skin of the stickleback in such great numbers that their substance forms a not inconsiderable fraction of that of the whole fish, it would have been difficult for them to have escaped me in my frequent examinations had they been present within the body of the fish. Everything speaks much more for the view that certain aquatic animals attach themselves in the spring to the skin of the stickleback, surround themselves with a cyst membrane, and in reproduction fall apart into the psorospermiform bodies. It is this animal which consists of a mucous substance, and which contains many scattered fat-like granules, and measures as much as 1''' long and about  $\frac{1}{2}$ ''' thick. The fat-like granules are employed in reproduction; they break up first into smaller parts and then form with a certain quantity of the structureless substance a globule which already constitutes the embryo of the new being. This grows gradually, one of the granules progressively increases in size and the remainder vanish. Growth then continues for a long time, until granules show themselves anew, which increase at the expense of the nucleus; the heretofore plainly visible surrounding membrane becomes apparently thinner or vanishes entirely, and thus a body is formed consisting of a mucous mass containing many small scattered granules and a nucleus [*Kern*] only a little larger, a body capable of motion and growth.

#### 14. Genus et sp. incert.

Psorosperms of *Leuciscus dobula*, Leydig, 1851, Müller's Archiv., p. 229.

Cyst not mentioned.

*Myxosporidium*.—Two or three spores develop in each pansporoblast (*Tochterblase*).

*Spore*.—Untailed.

*Habitat*.—On *Leuciscus (Squalius) cephalus* (= *dobula*).

#### 15. Genus et sp. incert.

Spores of *Squalius cephalus*, Schneider, 1875, Archiv. de Zool. Expér., Paris, IV, pp. 548-9.

Cyst and myxosporidium not mentioned.

*Spore*.—Capsules 2, with very long filaments, extruded under action of glycerin.

*Habitat*.—Air bladder of *Leuciscus (Squalius) cephalus*.

**16. Genus et sp. incert.**

Psorosperms of *Gobius fluviatilis*, Leydig, 1851, Müller's Archiv., p. 223, name only; *ib.* of *Gobio* [error] *fluviatilis* Ludwig, 1888, Jahresber. d. rhein. Fisch-Vereins, 1888, p. 30.

*Habitat*.—Body cavity of *Gobius fluviatilis* L.<sup>1</sup> (goby).

**17. Genus et sp. incert.**

Psorosperm of crocodile, Solger, 1877, Jahresber. schles. Gesellsch. f. Vaterl. Cultur, LIV, p. 45.

Name only, with statement that it will be fully described elsewhere.

*Habitat*.—In mucosa and muscularis of intestinal canal of "crocodile."

**18. Genus et sp. incert.**

Psorosperm of *Chondrostoma nasus*, Leydig, Müller's Archiv., 1851, p. 222.

No description or figure.

*Habitat*.—Cysts in roots of tongue of *Chondrostoma nasus* L.

**19. Genus et sp. incert.**

Psorosperms of *Leuciscus rutilus*, Leydig, Müller's Archiv., 1851, pp. 222-3.

No description or figure.

*Habitat*.—White clumps of "psorosperms" in the heart (auriculo-ventricular valve) of *Leuciscus rutilus*; also in heart blood of same fish.

**20. Genus et sp. incert.**

Psorosperms of *Cyprinus tinca*, Lieberkühn, 1854, Bull. Acad. Roy. Belg., XXI, pt. 2, p. 22.

No description.

*Habitat*.—Scales of *Tinca tinca* L. (tench).

**21. Genus et sp. incert.**

Psorosperms of *Cyprinus erythrophthalmus*, Lieberkühn, 1854, Bull. Acad. Roy. Belg., XXI, pt. 2, p. 22.

Mention of occurrence only; no description.

*Habitat*.—Subsquamous, on *Leuciscus* (*Scardinius*) *erythrophthalmus*.

**22. Genus et sp. incert.**

Psorosperms of *Gasterosteus aculeatus*, Hensen,<sup>2</sup> in Wittmack, 1875, Beiträge z. Fischerei-Statistik d. deutsch. Reichs, p. 190.

Mention only; no description.

*Habitat*.—On *Gasterosteus aculeatus* L. (stickleback) near Kiel.

**23. Genus et sp. incert.**

Psorosperms of *Lucioperca sandra*, Heckel & Kner, 1858, Die Süßwasserfische der österreichische Monarchie, Leipzig, p. 12; *ib.* Wittmack, 1875, Beiträge z. Fischerei-Statistik d. deutsch. Reichs, p. 190.

Heckel and Kner say:

Their gills are often beset with small cysts filled with a gelatinous fluid (the so-called psorosperms) and in this condition they are regarded as unfit for food.

<sup>1</sup>The great similarity of name between the present fish and *Gobio fluviatilis*, and the presence of a species upon the latter in the same situation (body cavity, see p. 243) suggests the possibility of an orthographic error.

<sup>2</sup>In response to an inquiry, Dr. Wittmack kindly informed me that Prof. Hensen's observation is unpublished, having been made upon a statistical question sheet.

I am indebted to the kindness of Dr. Wittmack for this reference.

*Habitat*.—Branchiæ of *Stizostedion lucioperca* (pike perch).

**24. Genus et sp. incert.**

Cyst of branchial "copules" of *Gasterosteus aculeatus* Thélohan, 1890, *Annal. de Microgr.*, II, p. 203.

No description.

*Effects*.—Pressure on the heart caused death.

*Habitat*.—Branchial "copulès" of *Gasterosteus aculeatus* (stickleback).

**25. Genus et sp. incert.**

Psorosperms of mackerel, v. d. Borne, 1886, *Handb. d. Fischzucht u. Fischerei*, p. 211.

No description (cf. p. 172).

*Habitat*.—On *Scomber scombrus* (mackerel).

**26. Gen. incert. ("Myxosporidium") bryozoides** Korotneff, 1892. Pls. 8, 9.

Korotneff's myxosporidian of <i>Aleyonella</i> <i>fungosa</i> .	bryozoides.	Date.	Authority; reference.
	Myxosporidium*.	1892	Ztschr. f. wiss. Zool., LIII, pp. 591-6, pl. 24, figs. 1-12.
	Do ....	1892	Henneguy & Thélohan, <i>Annal. de Microgr.</i> , IV, p. 617.
	Do ....	1893	Braun, <i>Centralbl. f. Bakt. u. Parasitenkde.</i> , XIII, p. 97.
x		1893	Ohlmacher, <i>Journ. Amer. Med. Assoc.</i> , XX, p. 562.
x		1893	Braun, <i>Centralbl. f. Bakt. u. Parasitenkde.</i> , XIV, p. 739.

*Myxosporidium* ? (*development of*).—For study of development, the polyzoan spermatoblasts offer a very rich material, comprising all stages of alterations. The earliest stage (pl. 9, fig. 1a) is a healthy, well-preserved cell, containing a large, round nucleus and, lying near it, the nucleus of the intruded myxosporidium, which latter is small, elongate-oval, dark-staining, and which, but for the complete series of changes exhibited by it, might be supposed to be a *Nebenkern*. The myxoplasm has, Korotneff inclines to believe, from the moment of its entrance so completely mixed with the polyzoan cytoplasm that we can no longer speak of a plasma differentiation.

The nucleus divides by mitosis (pl. 9, fig. 1b). Simultaneously or somewhat later the polyzoan cell-nucleus divides, but this latter division is never by mitosis, and is rather to be regarded as an externally induced fragmentation. The nonvital and artificial character of the cell-nucleus division is further shown by the variable size of the nuclei, resulting from the division, the nucleus having lost the capability of growth. Its division results from an irritation of, or better, an impulse from, the presence of the intruded myxosporidium. This artificial stimulation of the powers of the infected cell constitutes the peculiarity in the action of the parasite which thus prepares for itself an artificial ground without which its existence would be impossible. Sometimes cell-nucleus division takes place somewhat later than that of the parasite, so that we already find the parasite with 4 daughter nuclei (1 of which was

\* Name not in good standing (see p. 206).

seen in way of further division), the cell-nucleus being as yet unaltered. With continually progressing division, both of the myxosporidium and the cell nuclei, and with progressive growth of the cell body, the originally simple cell metamorphoses itself into a plasmodium. Thus a young plasmodium was seen in which 1 of the 2 daughter nuclei of the host-cell had fallen apart into 2 granddaughter nuclei, while the myxosporidian nuclei had in the same time increased much more. In the next developmental steps of the plasmodium the number of the nuclei increases very rapidly, and with such increase their energy becomes exhausted; the nucleoli vanish and the nuclear reticulum appears as a fine-grained granulation. Finally, the nuclear membrane shrinks and assumes an irregular contour. The cell nuclei then soon entirely vanish and we get a plasmode in which only myxosporidium nuclei are found.

With age the myxosporidia become displaced from the funicle and occupy the whole cavity. The zooid, thus become a myxosporidium-filled tube, closed at both ends. At this time the increasing mutual pressure produced by the continually growing myxosporidia results in their fusion to large plasmodes. Further growth produces rupture of the wall of the zooid and the myxosporidia come directly into contact with its chitinous investment.

The morphological characters of the adult myxosporidium are here interpolated.

*Myxosporidium?* (structure of adult).—Naked, membraneless, amœboid-variable, size 20 to 200  $\mu$ ; form varying greatly with age, the youngest being globular, the older ones oval or lobulated from adaptation to external pressure-conditions. Ectoplasm perfectly transparent and hyaline. Nuclei very numerous, consisting of clear round vesicles showing in the fresh state round nucleoli. Applied against the outside of (never within) each nucleolus is a small glittering globule. Pseudopodia formed by the ectoplasm, very fine, delicate and hair-like, ordinarily confined to a part and seldom covering the whole surface, often also forming small ramified tufts. Korotneff was unable to state whether the pseudopodia serve for attachment, but with the young myxosporidia the fixation to the funicle appeared really to occur through these structures.

Probably the direct influence of the water is injurious to them, and occasions a falling apart of the plasmodes and a freeing of the spores, which then fill the spongy chitin-masses of the atrophied colony. In this state the spores remain the whole winter, and in April follows, probably, the infection of the young *Aleyonella* (just out of the statoblast) by the amœba-brood from the spores.

The time of the appearance of the myxosporidia corresponds with the development of the spermatoblasts, which ordinarily begins (around Moscow) at the end of May, and the number of parasitic individuals increases *pari passu* with that of the spermatoblasts. While at the

first their existence is appreciable by the microscope, soon (July) they are visible to the naked eye, the lower end of the zooid tube losing its transparency and becoming milk white. In August the alteration becomes very marked, the cavity of the zooid being distended and completely opaque.

*Spore formation.*—How and whence do the spores originate? In any case their origin is endogenous (in the endoplasm) and probably occurs in the manner observed by Prof. Bütschli in *Myxidium lieberkühni*, where a spore membrane is formed around a trinucleate globule. In our case are often found, in the plasmodium, nuclei in state of division. Around such nuclei, which are still united by the threads of the spindle, a resistant shell appears often to be present. Could this be a spore? Korotneff is able to confirm Bütschli's observation that spore formation does not mark the end of the life cycle. In *M. bryozoides*, however, the spores always appear at a definite period of that cycle, viz, after the complete disappearance of the nuclei of the host-cell.

*Spore.*—Elongate-oval, resembling a melon seed, sharp anteriorly, rounded off posteriorly. Shell extremely hard, very resistant, lustrous, apparently with an opening at the sharp (anterior) end; no bivalve structure demonstrable, though empty spores are not rare. Often, but not always, two vacuoles are visible. In the spring he was able to distinguish at the anterior end of the spore a glittering point whose signification was unknown. It might possibly be a capsule (nematocyst; *Nesselkapsel*).

*Habitat.*—In very considerable numbers in the body cavity of *Acyonella fungosa* (a fresh-water polyzoan) in the neighborhood of Moscow, in the beginning of summer. The infection appears to be endemic, as Korotneff has never observed it in southern Russia and as it appears to be absent from western Europe.

*Seat and pathological anatomy.*—Principally grouped around the funicle upon which the spermatoblasts (which serve as food for the young myxosporidia) are produced. No tissue except the spermatoblasts is attacked. Repeated careful investigations showed the absence of myxosporidia from the polyp and from the walls of the zoecium.

*Effects.*—The extensive infection exerts a direct (but only a mechanical) influence on the polyp, producing, as a result of its continued growth, a progressive atrophy, which, by the end of August, results in the complete disappearance of the polyp. The infection extends itself through the colonies, scarcely a single zooid escaping. The death of the colonies occurs much earlier than it would naturally under the influence of cold.

*Remarks.*—Henneguy and Thélohan believe the reference of this form to the *Myxosporidia* absolutely justified, although the capsule has not been demonstrated.

## TRUE MYXOSPORIDIA.

Ordo I. *Cryptocystes* Gurley, 1893.

Etymology: *κρυπτος*, concealed, *κυστις*, capsule.

Bull. U. S. Fish Com. for 1891, xi, p. 409; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Myxosporidia* in which the pansporoblast produces many (8 or more) spores; the latter minute; without distinct symmetry; with but a single capsule; type (and only) family *Glugeidæ*.

## Fam. GLUGEIDÆ Gurley, 1893.

("Glugeidées" Thélohan, 1892, Bull. Soc. philomat. Paris, iv, pp. 173-4; *Glugeidea* [Thél.] Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, p. 739).

*Glugeidæ*, Bull. U. S. Fish Com. for 1891, xi, p. 409; *Glugeidæ* (error), Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Definition* (provisional as regards negative characters).—*Cryptocystes* destitute of a bivalve shell, with the capsule at the anterior extremity; an anidionophile vacuole; type genus *Glugea*.

This family now includes *Glugea*, *Pleistophora*, and *Thelohania*. Before the proposition of *Pleistophora*, only 2 genera had been proposed. Their distinction was practically based upon 3 characters, a comparison of which indicated very strongly that either there were too many genera or too few. If, as Henneguy and Thélohan and the writer believe, these characters are competent to determine generic lines at all (in the opposite case *cedit questio* and everything reduces to *Glugea*), then the spore of *Cottus scorpio* should form the type of a new genus, for (see table below) of the 3 characters but 1 is common to it and *Glugea*, and, although 2 are common to it and *Thelohania*, the third (divergent) character is one of no slight importance in *Thelohania*, as it is common to all the 3 (probably 4) typical species. For this genus I have proposed the name *Pleistophora*.

Myxosporidium.	Pansporoblast producing spores.	Pansporoblast membrane.	Genus.
Present.....	Inconstant, numerous....	Not subpersistent.....	<i>Glugea</i> .
Absent.....	Inconstant, numerous....	Subpersistent.....	<i>Pleistophora</i> .
Absent.....	Constant, 8.....	Subpersistent.....	<i>Thelohania</i> .

## I. GLUGEA Thélohan, 1891.

Etymology: Gluge.

Compt. Rend. hebdom. Soc. Biol. Paris, III, p. 29; *Gluega* [error] Thélohan, 1891, Compt. Rend. Acad. Sci. Paris, CXII, p. 171; *ib.* Thélohan, 1891, Journ. de Microgr., Paris, xv, p. 147; *Glugea* Thélohan, 1892, Bull. Soc. philomat. Paris, IV, p. 174; *ib.* Henneguy and Thélohan, 1892, Annal. de Microgr., IV, pp. 630, 636; *ib.* Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 409; *ib.* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkunde, XIV, p. 739; *ib.* Braun, 1894, *ibid.*, xv, p. 86.

**Definition.**—*Glugeidae* possessing a myxosporidium, and in which the pansporoblast produces an inconstant but large number (always more than 8) of spores; pansporoblast membrane not subpersistent; type, *G. microspora* Thél. (synonym for *anomala* Moniez).

27. *Glugea destruens* Thélohan, 1892.

Callionymus lyra, "corpuscles," etc., of	destruens.	Date.	Authority; reference.
×	.....	1891	Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, III, p. 28.
×	.....	1891	Thélohan, Compt. Rend. Acad. Sci. Paris, CXII, pp. 168-71.
×	.....	1891	Thélohan, Journ. de Microgr., XV, pp. 145-6.
×	.....	1891	Pfeiffer, Die Protozoen als Krankheitserreger, 2 ed., p. 115.
×	.....	1892	Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, IV, pp. 83-4.
.....	Glugea.	1892	Thélohan, Bull. Soc. philomat. Paris, IV, pp. 165, 174, footnote.
×	.....	1892	Henneguy & Thélohan, Annal. de Microgr., IV, pp. 618, 619, 636.
.....	Glugea.	1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, p. 409.
.....	Glugea.	1893	Braun, Centralbl. f. Bakt. u. Parasitenkunde, XIV, p. 739.
.....	Glugea.	1894	Braun, Centralbl. f. Bakt. u. Parasitenkunde, XV, p. 86.

Cyst none.

*Myxosporidium.*—Ectoplasm and endoplasm recognizable.

*Spore formation.*—Pansporoblast membrane thin, disappearing soon after spore formation. Sporoblasts, consisting of small globules with clear nuclei, sometimes disposed in very great numbers, sometimes isolated in groups of 4, 10, or 12 within the pansporoblast membrane.

*Spore.*—A little smaller than the similar parasite of *Cottus scorpio*, 2.5 to 3  $\mu$  long; 1 to 1.5  $\mu$  broad; characters otherwise identical (Thélohan, 1891). Length, 3 to 3.5  $\mu$ ; breadth, 2  $\mu$  (Thélohan, 1892, p. 174). Capsule present (Henneguy & Thélohan, p. 619).

*Habitat.*—Upon section of the muscles affected, the parasite is seen to have its seat in the interior of even the primitive fibrillæ of the muscles of *Callionymus lyra*. Not encysted, but forming a parasitic mass, destitute of an envelope, in which ripe spores are seen with others in course of development.

*Effects.*—Unlike the otherwise very similar condition in *Cottus scorpio*, the muscular fibers soon break up and undergo vitreous degeneration.

28. *Glugea anomala* Moniez, 1887. Plate 10, figs. 1-3.

Gasteros- teus acu- leatus, "corpus- cles," etc., of.	Pygos- teus pun- gitius, "corpus- cles," etc., of.	Aphya alba,* "para- site," etc., of.	anomala.	micro- spora.	Date.	Authority; reference.
×	-----	-----	-----	-----	1838	Gluge, Bull. Acad. Roy. Belg., V, pp. 772-6, figs. I, II.
×	-----	-----	-----	-----	1841	Gluge, Anatom.-micros. Untersuchgn. z. allgem. u. spec. Morphol., II, pl. 5, fig. 4 a-c.
×	-----	-----	-----	-----	1841	Müller, Müller's Archiv., p. 491.
-----	×	-----	-----	-----	1842	Creplin, Wiegman's Archiv. f. Naturgesch., I, pp. 64-5.
×	-----	-----	-----	-----	1843	Müller, Rayer's Archiv. de Méd. comp., I, pp. 266-268.
×	×	-----	-----	-----	1843	Rayer, Rayer's Archiv. de Méd. comp., I, pp. 266-70, pl. 9, figs. 11, 12.
cf.	-----	-----	-----	-----	1854	Lieberkühn, Müller's Archiv., pp. 9-12. (See also p. 183.)
-----	-----	-----	Nosema†	-----	1887	Moniez, Compt. Rend. Acad. Sci. Paris, CIV, p. 1312.
-----	-----	×	-----	-----	1888	Henneguy, Mém. publiées Soc. philomat. Paris, l'Occas. Centen. Fond., p. 170.
×	-----	-----	-----	-----	1889	Thélohan, Compt. Rend. Acad. Sci. Paris, CXI, p. 921.
×	×	×	-----	-----	1890	Thélohan, Annal. de Microgr., II, pp. 202-4, 211-12, pl. 1, figs. 4, 17.
-----	-----	×	-----	-----	1891	Garbini, Rend. Real. Accad. Lincei Roma, VII, Sem. 1, p. 153.
-----	-----	-----	Glugea	-----	1891	Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, III, p. 29.
-----	-----	-----	Glugea	-----	1891	Compt. Rend. Acad. Sci. Paris, CXII, p. 170.
-----	-----	-----	Glugea	-----	1891	Thélohan, Journ. de Microgr., XV, p. 147.
-----	-----	-----	Glugea	-----	1892	Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, IV, pp. 82-4.
-----	-----	-----	Glugea	-----	1892	Thélohan, Bull. Soc. philomat. Paris, IV, pp. 165, 174.
-----	-----	-----	Glugea	-----	1892	Henneguy and Thélohan, Annal. de Microgr., IV, pp. 619, 631, 633-6.
-----	-----	-----	Glugea	-----	1893	Braun, Centralbl. f. Bakt. u. Parasitenkde, XIII, p. 96.
-----	-----	-----	Glugea	-----	1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, p. 409.
-----	-----	-----	Glugea	-----	1893	Braun, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739.
-----	-----	-----	Glugea	-----	1894	Braun, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 86.

\* The species is (*vide* Henneguy, letter to author, 1893) *Gobius albus*. This identification was made by a "specialist." Dr. Gill informs me that the name *Aphya alba* should be used.

† *Nosema* Nägeli, 1857, was founded upon *N. bombycis* Nägeli, which was regarded as a Schizomycete (Tagebl. 33 Versamml. deutsche Naturf. u. Aerzte, im Bonn, 1857, p. 27).

*Cyst development.*<sup>1</sup>—In a *G. aculeatus* kept under observation for nearly a year there existed at first a single cyst, quite regularly spherical, attaining nearly the volume of a pea. Very soon small secondary vesicles, at first scarcely distinct, appeared upon its surface, progressively enlarged and finally, instead of the primary cyst shelling out as a whole, it split open at the most prominent point and a great part of its contents escaped, leaving in place of the tumor an excavation irregularly limited by a ridge formed by the non-empty part of the small sphere. The small secondary vesicles then developed rapidly and very soon formed an irregular strawberry-like mass.

<sup>1</sup>Thélohan (Annal. de Microgr., 1890, II, p. 204; Compt. Rend. hebdom. Soc. Biol. Paris, 1892, IV, p. 82) also saw cysts enlarge, become subcutaneous, shell out from their attachments into the water, and there burst.



*Cyst structure.*<sup>1</sup>—Number, 1 to 4 (sometimes a dozen, Thélohan), rarely more, in contact or more or less widely separate; the majority as large as a small pea, some, however, attaining only the size of a pin's head; size of tumor bearing no relation to that of the fish, being variable in the same individual; shape regularly spherical or only a little rounded; color usually whitish—when covered by the epidermis of the fish, silvery. Membrane always present, resistant, usually covered by the epidermis, which forms an outer cyst; surface granulated by alcohol; Contents consisting of a small quantity of a colorless fluid coagulable by alcohol, holding in suspension immense numbers of corpuscles which yield bubbles of gas (CO<sub>2</sub>?) with mineral acids. Müller (1841, p. 491) found also some microscopic crystals. Thélohan (1890, p. 204) adds that the average thickness is 5  $\mu$ ; under high powers the membrane shows a fibrillary structure parallel to the surface of the cyst. Thélohan believes the membrane to be nonnucleated and considers this a strong argument in favor of its derivation from the similarly nonnucleated myxosporidian ectoplasm.

*Myxosporidium.*—Spore formation:<sup>2</sup> Myxoplasm containing small nucleated globules which surround themselves with a thin membrane, divide, and end by forming small spheres filled with very numerous rounded nucleated elements which later will yield the spores.

*Spore.*—Very numerous, transparent, regularly ovoid, 3 to 5  $\mu$ -long, 2 to 3  $\mu$  broad, size and form constant in spores from the larger cysts, less clear in those from the smaller. Shell bivalve; structure not demonstrable; chemical characters the same as those of other spores. Interior of spore showing a shaded portion at the smaller, and a clear portion filling the larger, extremity. Capsule 1, filament very long (50  $\mu$ ), extruded under the influence of iodine. No other reagent produced such extrusion. The central (iodinophile) vacuole appears to be absent; a vacuole uncolorable by iodine is present, however, usually in the larger end, less frequently subcentral. Thélohan (1890, p. 212) has traced the division of the nuclei up to 4, a number which he has never seen (but which he does not wish to assert may not be) exceeded.

*Micro-chemistry.*—Acetic acid produces no change. Sulphuric acid causes evolution of bubbles of gas (CO<sub>2</sub>?), the corpuscles at the same time becoming less clear but not dissolving. Potassium hydrate causes an agglomeration similar to the "rouleaux" of blood corpuscles (Gluge). The best stains for this species, Thélohan found to be gentian violet; but above all, safranin by the Gram-Bizzozero method.

*Habitat.*—Subcutaneous cysts of *Gasterosteus aculeatus* (stickleback) in European rivers, occurring only once in every 20 or 30 fishes examined (Müller). Subcutaneous cysts of *Pygosteus pungitius* (9-spined stickle-

<sup>1</sup> Description Gluge's unless otherwise stated.

<sup>2</sup> Thélohan's observations on a myxosporidium in *G. aculeatus* (Journ. de Microgr., 1891, xv, p. 147).

back. The forms habitant on these 2 fishes are identical, differing only a little in the size of the cysts (all *vide* Thélohan). Subcutaneous cysts of *Aphya alba* (= *Gobius minutus* and *G. albus*). In the last the deformity is even greater than in *G. aculeatus*.

*Nature*.—For Gluge's opinion, see p. 93.

*Effects*.—Even where the tumors occupy the internal surface of the opercle the fish did not appear to be hampered in its functions. Those which carry the tumors on the fins, nevertheless move the latter as freely and actively and execute all movements with the same facility as the sticklebacks not so affected. The tumors may be carefully removed without injuring the fish, which appears as well as ever after the operation. Upon careful dissection, Gluge was unable to find any change in the intestine or in the blood. Thélohan (1890, p. 203) states that in certain cases the muscles are compressed and atrophied by pressure of the tumors, and the viscera are also compressed and no longer present their normal position or relations.

## II. PLEISTOPHORA Gurley, 1893.

Etymology: *πλειστος*, very many; *φέρειν*, to carry.

Bull. U. S. Fish. Com. for 1891, XI, pp. 409, 410; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Definition* (provisional as regards negative characters).—*Glugeidæ* destitute of a myxosporidium and in which the pansporoblast produces an inconstant but large number (always more than 8) of spores; pansporoblast membrane subpersistent as a polysporophorous vesicle; type, *P. typicalis*.

### 29. *Pleistophora typicalis* Gurley, 1893.

(Corpuscles of *Cottus scorpio* Thélohan, 1890, Annal. de Microgr., II, pp. 203, 212; *ib.* Thélohan, 1891, Journ. de Microgr., xv, pp. 145, 146; *ib.* Thélohan, 1891, Compt. Rend. hebdom. Soc. Biol. Paris, III, pp. 27, 28; *ib.* of *Collus* (error) Thélohan, 1891, Compt. Rend. Acad. Sci. Paris, CXII, p. 170; *ib.* Pfeiffer, Die Protozoen als Krankheitserreger, 2 ed., pp. 113–115; *ib.* Thélohan, 1892, Compt. Rend. hebdom. Soc. Biol. Paris, IV, pp. 82, 83; *ib.* Thélohan & Henneguy, 1892, *ibid.*, p. 586; *ib.* Thélohan, 1892, Bull. Soc. philomat. Paris, IV, pp. 165, 174; *ib.* Henneguy & Thélohan, 1892, Annal. de Microgr., IV, pp. 618, 619, 622, 631, 636.)

*Pleistophora typicalis*, Bull. U. S. Fish Com. for 1891, XI, p. 410; *ib.* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Cyst*.—None.

*Spore formation*.—Thélohan observed between the fibrillæ small separate masses of protoplasm, each with a distinct membrane and nuclei. These masses were  $4\mu^1$  long by 2.5 to  $3\mu$  broad. Thélohan believed them to represent the first stages of development, but emitted this opinion with reserve, not having seen a sufficient series of stages. Some protoplasmic masses inclosing several nuclei exhibit, however, intermediate stages between the masses already described and the pansporoblasts.

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<sup>1</sup>"4 Cent." in Journ. de Microgr., xv, p. 146.

Pansporoblast spherical, average diameter 15 to 18  $\mu$ ; membrane thin, transparent, containing, besides fully developed spores, sporoblasts in different stages of development, some of them measuring 2.5 to 3  $\mu$ , and containing one or several colored granules representing nuclei.

*Spore*.—Ovoid, resembling that of *Glugea anomala*; length, 3  $\mu$ ; breadth, 1.5 to 2  $\mu$ ; a single capsule with a filament is present; large extremity showing a mass refractory to staining fluids, the remainder of the spore cavity containing sporoplasm, and a body apparently representing the nuclear element of the spore, staining strongly with reagents, and in certain cases decomposable into separate granules whose number never exceeds 4.

*Habitat*.—Muscles of *Cottus scorpio* (sculpin); position interfibrillar.

*Effects*.—Diseased mass forming small white streaks of an average size of 5 to 6 mm. by 3 mm., consisting of spores. The fibers affected increase in bulk; they are filled with the pansporoblasts disposed without regular order between the fibrillæ, which latter become separated and distorted, without, however, presenting any alteration of structure or diminution in the clearness of their transverse striation.

### III. THELOHANIA Henneguy, 1892.

Etymology: Thélohan.

In Thélohan, Bull. Soc. philomat. Paris, iv, p. 174, footnote; *ib.* Henneguy, in Henneguy and Thélohan, Annal. de Microgr., Paris, 1892, iv, p. 639; *ib.* Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, pp. 409-410; *ib.* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, pp. 739-740; *ib.* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Definition*<sup>1</sup> (provisional as regards negative characters).—*Glugeidae* destitute of a myxosporidium and in which the pansporoblast produces constantly 8 spores; pansporoblast membrane subpersistent as an octosporophorous vesicle; type *T. giardi*.<sup>2</sup>

Henneguy and Thélohan remark that in this genus the spores unquestionably approximate those of *Glugea anomala* and those of *Pleistophora*. The number of spores formed in the pansporoblast and the absence of a myxosporidium differentiate *Thelohania* from *Glugea*. On the contrary, the last character and the subpersistence of the pansporoblast membrane as a sporophorous vesicle, approximate it to *Pleistophora*.

<sup>1</sup> Henneguy's definition is:

"Spores pyriform, with one polar capsule at the small extremity and, at the opposite extremity, a clear vacuole with contents not colorable by iodine. Sporoblasts producing only 8 spores surrounded by an envelope persisting after the formation of these last; no plasmic mass, properly speaking."

As constituted by Henneguy the genus included only 3 species, *T. octospora*, *T. giardi* and *T. contejeani*.

<sup>2</sup> Type proposed by the author in Bull. U. S. Fish Com. for 1891 (1893), xi, p. 410.

**30. *Thelohania contejeani* Henneguy, 1892. Pl. 10, figs. 4, 5.**

'Parasite of crayfish, Henneguy and Thélohan, 1892, *Compt. Rend. hebdom. Soc. Biol. Paris*, iv, p. 749.)

*Thelohania contejeani*, in Thélohan, *Bull. Soc. philomat. Paris*, iv, p. 174, footnote; *ib.*, Henneguy and Thélohan, 1892, *Annal. de Microgr.*, iv, pp. 637-9, pl. 4, figs. 26-7; *ib.*, Braun, 1893, *Centralbl. f. Bakt. u. Parasitenkde*, xiv, pp. 739-740; *ib.*, Dubois<sup>1</sup> (Raphæl) 1893, *Recherches de pathologie comparée sur la peste des écrevisses*, *Compt. Rend. hebdom. Soc. Biol. Paris*, v, pp. 158-9, figs. A, B; *ib.*, Gurley, 1893, *Bull. U. S. Fish Com. for 1891*, xi, p. 410; *ib.*, Braun, 1894, *Centralbl. f. Bakt. u. Parasitenkde*, xv, p. 86; cf. *La Maladie des Écrevisses en Allemagne*; *Bull. Mensuel Soc. Nat. d'Acclimat. France*, February, 1884, p. 200 (transl., *Bull. U. S. Fish Com. for 1884*, iv, pp. 299-302).

*Cyst.*—None. Parasitic mass producing an opacity of the affected muscles, as in *Palæmon* and *Crangon*. Opacity more difficult of observation than in the last, on account of the greater thickness of the test; easily detected, however, on the inferior surface of the abdomen.

*Adult.*—In some places only spores are seen; in others small plasmaspheres, containing a variable number of nuclei, occur. These are evidently developmental stages, but a full series could not be found.

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<sup>1</sup>This observer noted 2 (entirely distinct) parasites, viz: one which Henneguy and Thélohan pronounced a fungus, and one which he determined to be *Thelohania contejeani*.

1. The former he describes as follows:

*Spore.*—Cells elongate, ovoid, cylindrical, or strangulated toward the middle, according to the degree of development. Shell double-contoured; protoplasm vacuolate, escaping amœboidly through a small lateral orifice. Spores apparently not capable of growth in nutritive fluids.

*Habitat.*—Confined to the intestinal canal of the diseased crayfishes. The observations were made in June and July (1892), the months of maximum severity of the epidemic.

*Crayfish epidemic.*—Causes: Alterations of streams by industrial or agricultural products can have only a subordinate and local influence.

Area invaded divisible into 3 zones: (1) Lake Mantua (and its outlet to the sea, the river Ain); formerly renowned for its crayfishes, which constituted an important revenue; now destitute of crayfishes. (2) The Merloz rivulet, an affluent of the lake, containing sound and diseased crayfishes, the latter showing the symptoms of the pest. (3) The sources or *Doye des Neyrolles* feeding the lake and the Merloz rivulet, from which latter it is separated by a dam, above which all the crayfishes are healthy.

The stoppage of its advance by the dam and its inability to grow in nutritive fluids caused Dubois to suspect it to be an animal (possibly a sporozoan) which ascended the watercourse from the sea, perhaps brought by a fish. Thélohan and Henneguy, however, from an examination of his material, believed the form to be a fungus.

The Distome described by Baer in 1827 (when no epidemic existed), to which Harz attributes the crayfish epidemic, was sought for in vain.

2. *Thelohania contejeani*.—Feeding experiment: Sound crayfishes were isolated in reservoirs and fed, some with butcher's meat, and others with the flesh of trout, carp, pike, and roach. After three months these fed on roach showed parasites in the abdominal muscles. This parasite was identical with *Thelohania contejeani*. Dubois asks: Do relations exist between the parasite found in the muscles and the intestines in October, and that found in July in the abdomen?

*Spore formation.*—Number of spores found in each sporigenous area variable, always, however, more than 8, in which respect the present species differs from the spores of *Palæmon* and *Crangon*.<sup>1</sup> Spores sometimes free, sometimes 8 together in a common envelope, as in *Palæmon*.<sup>2</sup>

*Spore.*—Size approaching and appearance the same as that of *T. octospora*; ovoid, length 2 to 3  $\mu$ , with a clear vacuole in the larger end.

*Habitat.*—Striated muscles of *Astacus fluviatilis* (crayfish) from the Department of Doubs, France; collected by M. Contejean in 1890.

*Pathological anatomy.*—On section the muscles show nearly the same appearance as in *Palæmon* and *Crangon*; the fibrillæ being separated by parasitic masses, which in transverse sections appear as numerous deeply stained punctules, and which in longitudinal sections assume the appearance of irregular chains separating the fibrillæ; the latter have preserved their normal appearance, the striæ being perfectly distinct.

*Nature.*—The material was available only in alcohol, to which it had been transferred from Fol's liquid. Owing to this, Henneguy and Thélohan were unable to demonstrate the capsule with filament. The similarity to the other species leads them, however, to believe it a myxosporidian.

*Effects.*—A notable diminution of muscular vigor was clearly established with the myograph by M. Contejean.

*Epidemics.*—In the Department of Doubs this disease has raged with intensity among the crayfishes during several years and has caused the death of a very great number of individuals. It seems now to have disappeared. Moreover, this parasite can hardly be special to the watercourses of Doubs, and, remembering the considerable mortality caused by it in that Department, it is to be presumed that this hitherto unknown organism has played a rôle in the genesis of the epidemic which raged for several years in the East, and which has almost completely destroyed the crayfishes of that region.

31. *Thelohanias octospora* Henneguy, 1892. Pl. 10, fig. 6; pl. 11, figs. 1-5.

(Parasite of *Palæmon rectirostris* and of *P. serratus*, Henneguy, 1888, Mém. publiées Soc. philomat. Paris l'Occas. Centen. Fondation, pp. 163-71; *ib.*, Thélohan, 1891, Journ. de Microgr., xv, p. 146; *ib.* of *P. rectirostris*, Thélohan, 1891, Compt. Rend. hebdom. Soc. Biol. Paris, III, p. 28, name only; *ib.*, Thélohan, 1891, Journ. de Microgr., xv, pp. 146-7; *ib.*, Pfeiffer, 1891, Die Protozoen als Krankheitserreger, 2 ed., pp. 114-5; *ib.*, Thélohan and Henneguy, 1892, Compt. Rend. hebdom. Soc. Biol. Paris, IV, p. 586.)

*Thelohanias octospora* in Thélohan, Bull. Soc. philomat. Paris, IV, pp. 165-6, 174, footnote; *ib.*, Henneguy and Thélohan, 1892, Annal. de Microgr., IV, pp. 621-27, 629-632, pl. 4, figs. 1-8; *ib.* Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 410; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 739-40; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

<sup>1</sup>Henneguy and Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, 1892, IV, p. 749.

<sup>2</sup>Henneguy and Thélohan, 1892, Annal. de Microgr., II, p. 638.

*Life history.*—All the individuals, whether wholly or only partly invaded, showed the same developmental stage. It seems fair to suppose the first stage to be a plasmodioid mass in which the spores form. The constant presence of 8 spores suggests their origin by successive bipartition, as occurs with the falciform corpuscles of Gregarines (Henneguy, 1888). The stage of development of the parasite of *P. serratus*, taken in connection with the date of capture, indicates that the course of development of the parasite is the same in this crustacean as in *P. rectirostris* (Henneguy and Thélohan, 1892).

*Cyst.*—Henneguy vainly endeavored to detect, even under very high powers and with different reagents, in material, fresh or fixed, dissociated or sectioned, a cyst membrane, and believes the cyst to be absent. This view is, he thinks, confirmed by the irregularity of the distribution of the pansporoblasts between the fibrillæ.

*Pansporoblast* ("vesicles" of Henneguy, 1888).—Rounded, diameter,  $10\ \mu$ ; membrane thin, transparent, resisting potassium hydrate solution, apparently not presenting local thickenings as in *T. giardi*.

*Spore formation.*—Each pansporoblast produces 8 spores, which fill only a portion of its cavity and are disposed without order.

*Spore.*—Length, 3 to  $4\ \mu$ ; pyriform, very refringent; capsule present; length of filament 40 to  $50\ \mu$ ; exit, produced, after failure of all other reagents, by ether, whose action is rapid and perfectly definite, and affects a large number of spores; usually extruded completely, sometimes, however, only partially uncoiled; capable of staining with anilin stains, among others violet 5B. The electivity of the filament for ether is a striking peculiarity.

*Habitat.*—Interior of muscular fibers (between the ultimate fibrillæ) of *Palaemon rectirostris* Zadd (prawn), from the salt marshes at Le Croisic; the same seat in *P. serratus* from Concarneau and from Roscoff. In *P. serratus* less common than in *P. rectirostris*, in which latter it is (at least at Le Croisic) extremely frequent. It is never found in the digestive tract, nervous system, glands, sexual organs, or anywhere but in the muscles.

*Affinities.*—By its exclusive seat in the muscles, and by the form and grouping of the spores, the parasite appears to be incontestably a sarcosporidian, differing from those of the *Mammalia* in the absence of a surrounding membrane. The spores, also, are a little different from those of the other *Sarcosporidia*. They recall certain myxosporidian spores. This form also presents much affinity with the *Microsporidia* of the *Arthropoda*, the latter having the same refringent aspect and more or less oval shape of the present species, and being, like it, inclosed in "vesicles." One finds them in all tissues, but not in the interior of the muscle fiber. There, then, probably exists a rather close relation between the *Micro-*, *Myxo-*, and *Sarcosporidia*, and the parasite of *Palaemon* appears to represent a transition form between the 3 groups (Henneguy, 1888).

The discovery of the capsule settles the question in favor of its myxosporidian nature. It is thus neither a sarcosporidian nor a transitional form (Henneguy and Thélohan, 1892).

*Microscopic technique*.—Henneguy fixed by alcohol, osmic acid solution, Flemming's, Perenyi's, or Kleinenberg's liquids, dehydrated, paraffined, sectioned, affixed with Mayer's albumen, and stained, preferably with gentian violet (Ehrlich's) and eosin. Parasites (also nuclei of muscles, connective tissue, epithelia, nerves; which, however, can be washed out) violet; muscles rose-red. Picro-carmin; muscles red, spores yellow. Safranin; tissue nuclei red, spores same, but fainter.

*T. octospora* differs from *T. giardi* in the smaller size of the pansporoblast, and apparently also in the absence of thickening of its membrane.

*Pathological anatomy*.—Macroscopic: Easily recognizable by the chalky or porcelainous opacity<sup>1</sup> which forms a constant and characteristic sign of the presence of these *Myxosporidia*. Opacity limited to the muscles invaded, consequently varying in extent with the degree of infection; in slight (and in the beginning of all) cases being limited to some white striæ in one or several abdominal segments, or only one or two segments (most frequently then the first ones, the disease appearing to progress from before backwards) are opaque white. *Ad maximum*, the entire body becomes white except the region of the heart and stomach which always, and some parts of the claws, antennæ, beak, and abdominal segments which usually, remain transparent. These exceptions constitute the only difference between this condition and the opacity produced by heat or alcohol.

*Microscopic*.—Low powers: In examining a teased or slightly compressed muscle fragment, one immediately perceives, besides the normal primitive fiber bundles (easily recognizable by their transverse striation), elongated spaces parallel to these bundles, contrasting strongly therewith, and apparently filled with a peculiar finely granular substance. Dimensions of spaces approximating those of the normal fiber bundles; their transverse diameter, however, a little greater. Number of spaces varying *pari passu*, and the intervening sound tissue varying inversely, with the intensity of the infection, the opaque spaces being in contact or more or less widely separated by sound fiber bundles. The proportion of the fibrillæ invaded is best appreciated in transverse sections of the muscles. In extreme cases nearly all the fibers may be affected. Longitudinal sections show the parasite in the form of violet chains between the rose-red normal fibrillæ (gentian violet; safranin).

Higher powers: At first sight one would believe that each of these productions is entirely composed of a parasitic mass interposed between the primitive fibers, but a more thorough examination shows

<sup>1</sup>The same opacity is found in the muscles of *Callionymus lyra*, *Cottus scorpio*, and *Barbus barbus*, and outside the muscles the parasites exhibit the same color.

that each space corresponds to a primitive fiber bundle whose normal aspect is profoundly modified by the presence between its fibrillæ of elements of a parasitic nature, whence results a slight increase of width of the fiber bundle. Most often the fibrillæ do not present a sensible alteration. Sometimes (probably when a great quantity of the parasitic element has led to a considerable separation) the elasticity of the fibrillæ is overcome, rupture resulting. Even under these conditions, however, the muscle striæ remain exceedingly clear, no degeneration ever having been observed, as in *Callionymus* and the barbel.

The nuclei of the muscle fiber are more numerous and smaller than normal; this feature is particularly well shown by safranin (Henneguy, 1888).

*Effects.*—The muscular vigor is considerably diminished. Thus, if a number of *P. rectirostris* living in the rivulets of the salt marshes be frightened out of their shelter among the vegetation, even although the new shelter sought by them be near at hand, the diseased white individuals (immediately recognizable against the strongly contrasted muddy rivulet bottom) lose ground and remain considerably behind the sound ones. Further, one knows with what ease the prawns jump out of the vase in which they are held captive. If sound and opaque prawns be placed together in a basin, after some hours the sound ones have nearly all dispersed around the vessel, while the opaque are there still, or have only succeeded in sticking to the wall of the basin, however small the bound required to overleap the barrier. Considering the intensity and universality of the muscle infection, the diminution of muscular vigor is quite natural; indeed, the surprising feature is the relatively great agility retained by muscles the bulk of whose contractile substance is much inferior to that of the parasite, and in some cases it is truly astonishing that muscular power is not completely destroyed. Among the diseased *Palaemons* no egg-bearing females were seen. Perhaps this may be a case of "parasitic castration." The diseased individuals do not survive very long, all succumbing by the end of autumn, as during the winter not one can be found.

*Conditions and mode of infection.*—The prawns affected are usually found in small shallow ditches containing a layer of water 0.10 m. to 0.20 m. deep, along the slope separating the compartments from the salt marshes. The water of these ditches is rarely renewed and acquires an elevated temperature. These are probably the conditions favorable to the development of the parasite. It is difficult to decide whether the parasite finds an entrance by way of the alimentary canal. Henneguy seems to favor the contrary view, as the first lesions are found at places remote from the digestive tract.

*Artificial infection.*—Captive *Palaemons* fed for several months with diseased tissue showed no signs of infection. It was impossible to prolong the experiment to see whether infection would ultimately ensue (Henneguy, 1888). *P. rectirostris* fed for months with diseased tissue



never showed, under the most careful microscopic examination, the slightest trace of infection (Henneguy and Thélohan, 1892).

*Season.*—Disease most frequent and at maximum of development from about July 15 to the end of August; number affected diminishing in September; diminution more pronounced in October; disappearing entirely after November 15; reappearing about March 15 or the first days of April.

32. *Thelohania giardi* Henneguy, 1892. Pl. 12, figs. 1, 2.

Crangon vulgaris, "parasite," etc., of.	giardi.	Date.	Authority; reference.
×	-----	1892	Thélohan & Henneguy, Compt. Rend. hebdom. Soc. Biol. Paris, IV, pp. 586-7.
	Thelohania .	1892	Henneguy in Thélohan, Bull. Soc. philomat. Paris, IV, pp. 165, 174, footnote.
	Thelohania .	1892	Henneguy & Thélohan, Annal. de Microgr., IV, pp. 621, 624, 626-31, pl. 4, figs. 9-25.
×		1893	Ohlmacher, Journ. Amer. Med. Assoc., XX, p. 562.
	Thelohania .	1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, p. 410.
	Thelohania .	1893	Brann, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 739-740.
	Thelohania .	1894	Braun, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 86.

\* Crangnon; error.

*Cyst unknown.*

*Spore formation.*—Pansporoblast spherical; diameter  $14\ \mu$  (12 to  $14\ \mu$ ); in the young stages consisting of a very thin membrane resisting potassium hydrate, inclosing a very transparent, scarcely granular, slightly refringent protoplasm, having at its center a rather large nucleus (pl. 12, fig. 1a, b), often visible in the fresh state, becoming much clearer under the action of reagents.

(1) Segmentation of the pansporoblast: The nucleus first presents the typical resting structure with a distinct membrane. The chromatin can take on different arrangements, sometimes forming one grain much larger than the others, sometimes a variable number of smaller sub-equal grains, or sometimes crowded back against the membrane, presenting here and there thicker portions (pl. 12, fig. 1). Subsequently a remarkable modification occurs: the chromatin has become arranged in filaments, the membrane has disappeared, and the nucleus assumes the arrangement known as the chromatic coil; very soon the chromatic filaments orient themselves into a very distinct equatorial plate, which becomes double, the process resulting in the formation of 2 daughter-nuclei. We thus have a true karyodieresis. The achromatic filaments were not seen, doubtless owing to their rather small size and partly, Henneguy and Thélohan believe, to the nature and optical properties of the protoplasm. Protoplasmic segmentation soon follows nuclear division, and one sees, within the primitive pansporoblast membrane, 2 small distinct nucleated masses. In their turn these 2 masses divide and redivide, the process ending with the formation of 8 small plasmic bodies (*sporoblasts*) within the original pansporoblast membrane. The divisions do not take place very rapidly, and between successive ones

the nuclei have time to return to a state of rest, whence they again pass through the same stages preliminary to division.

The sporoblasts have no regular arrangement within the pansporoblast membrane; their shape is inconstant, varying with their arrangement; they generally approximate a truncate-pyramidal form. Each sporoblast develops into a spore. Spores thus contained 8 in each pansporoblast membrane, without regular arrangement, not nearly filling the cavity. This is the last stage of development reached in the muscles of the host.

Pansporoblast membrane retaining its original dimensions, perfectly transparent, very thin, although the double contour is easily visible, showing in optical section marked thickenings, often 2 in number (pl. 12, fig. 1*k*).

(2) Development of sporoblast into spore: Owing to the very minute size of these bodies, it is almost impossible to follow this development in detail or to confirm the facts discovered in the larger forms by Thélohan, viz, sporoblast segmentation, number of nuclei, etc.

Development of capsule: A peculiar arrangement, believed to be connected with the development of the capsule, was noted, viz: often in the body of the sporoblast, near the nucleus, a clear rounded space, into which a small protoplasmic button projects. This observation is, however, a very delicate one, and the figures are slightly diagrammatic.

*Morphology of the sporophorous vesicles.*—The constitution and development of the spore-producing vesicles permit us to consider them only as the morphological equivalent of the pansporoblasts of the other *Myxosporidia*. These octosporophorous pansporoblasts form a transition from the oligosporogenetic pansporoblasts of the larger species to the polysporogenetic pansporoblasts of *Glugea*, which latter produce a considerable and inconstant number of spores. Above all, one fact is here to be noted, viz, the entire absence of a myxosporidium. No structure whatever could be detected which could be regarded as its morphological or physiological equivalent.

But whence come these spore-producing vesicles? Evidently they do not represent the first stage of development. Now if, as is usual, they are formed in the interior of a protoplasmic mass, what has become of the latter? In all other known species a considerable protoplasmic residue remains, even of myxosporidia whose development is completed, and in which young pansporoblasts are no longer to be found, but only entirely mature spores. But here are young pansporoblasts at their simplest (uninucleate spherules) with not the slightest trace of a surrounding protoplasm. As long as we had only found these organisms in the mature state (as sporophorous vesicles) that absence might have been explained, in case of necessity, on the supposition of a complete previous transformation of the myxosporidium into pansporoblasts, the myxosporidium vanishing in the process or leaving only insignificant vestiges. But in the presence of the now known earlier phases of development this hypothesis seems hardly admissible.

Henneguy and Thélohan add:

Is it necessary to admit the existence of a plasmic mass [myxosporidium] which is completely transformed into sporoblasts? This mode of view can evidently be defended; no fact, however, comes to its support, and it has the grave fault of deviating widely from what one knows of the development of the other species. On the whole we must admit that there is here a point in the history of our parasite which our researches have not elucidated, and the state under which it is presented constitutes a curious peculiarity which, at least in appearance, establishes an important distinction between it and the other *Myxosporidia*.

*Abnormalities of development.*—One rather frequently encounters spores which are larger than the others and which exhibit a constriction (pl. 12, fig. 11). At first view one is tempted to question whether this is not a phase of division. Similar productions are rather frequent in *Glugea* and in the *Microsporidia* (whose spores offer much resemblance to those of *Thelohania*), where they have been seen by Pasteur,<sup>1</sup> who considered them as corpuscles in process of division. On the contrary, Balbiani, who has studied them with care, regards them as the result of malformations, a view which Henneguy and Thélohan adopt in the present species. If fig. 12, pl. 11, be considered, it is quickly seen that this is the only interpretation admissible. One sees there 4 normal spores, and 2 larger structures constricted toward their middle and presenting attenuated extremities similar to the small ends of normal spores. The appearance of these elements and their dimensions cause one to think of 2 spores soldered by their large extremities. There can no longer remain any doubt in this respect if one considers that by supposing these spores separated the typical number of spores in the pansporoblast is made up. In reality, then, the 2 spores in question have, in consequence of an accident which has occurred in the course of their development and by a process which we have not been able to follow, contracted an intimate adhesion at the level of their large extremity, the point where this soldering has taken place remaining marked by a constriction. The limited number of spores in each pansporoblast renders the proof much more easy here than in *Glugea* and the *Microsporidia*, where the number of spores is much greater and not constant.

[I can not see why these could not be more simply and better explained as malformations, the result of development from imperfectly segmented pansporoblasts, i. e., as developing from a quarter-segment of the pansporoblast which failed to divide completely. The partial fusion of 2 spores where no pressure-atrophy of the shell could be assumed, seems very improbable. (cf. p. 180). R. R. G.]

Finally, although not pertaining directly to the *Myxosporidia*, in this connection the following from Kunstler and Pitres<sup>2</sup> may be quoted:

The small forms often show themselves constituted in such a manner that they appear to be in way of division (figs. 8-12). The multiplicity, the variety, and the constancy which these appearances present seem to show well that this is really a

<sup>1</sup> Études sur les maladies des vers à soie, Paris, 1870.

<sup>2</sup> Journ. de Microgr., 1884, VIII, p. 522.

process of division. Some divide into 2 equal parts (fig. 8); in others the parts are of unequal dimensions (figs. 9, 10), and often this division recalls strongly a phenomenon of terminal or lateral budding (fig. 11).

*Spore*.—Very refringent, pyriform; anterior end much more acute; length 5 to 6  $\mu$ ; shell with very fine longitudinal striæ; could not determine whether bivalve or not.

*Capsule*: In fresh material the highest powers reveal nothing suggestive of a capsule, the anterior extremity appearing merely more shaded, seemingly occupied by a homogeneous, refringent substance. One sometimes sees, however, near the anterior end, a clear streak (pl. 12, fig. 10) believed to be due to the capsule, but it is too indefinite and exceptional to prove the existence of that structure. Stained sections afford no aid here.

*Filaments*: Extrusion not produced by iodine, potassium or sodium hydrates, glycerin, heat, acetic or formic acids, or by ether. Hydrochloric and nitric acids produced extrusion; the latter difficultly obtainable, observed only in a very small number of cases in spite of repeated efforts. Strangely enough, this method failed completely to produce extrusion in *T. octospora* and, on the contrary, ether, the only agent which succeeded in that species, was without effect on the spores of *T. giardi*. Filament 15 to 20  $\mu$  long; usually extruded completely, sometimes, however, extruded only partially uncoiled; susceptible to anilin stains, among others violet 5B.

*Sporoplasm*: Safranin or gentian violet (apparently the best stains for these organisms) yield 2 different appearances, according to the degree of decoloration. If slightly decolorized, the vacuole alone is visible, but when decolorized *ad maximum* only some colored grains remain in front of the vacuole. Sometimes two or three are distinguishable; most frequently, however, only a small colored band (apparently formed of fused granules of indeterminate number) is seen. Vacuole aniodinophile.

*Habitat*.—Seen only once in *Crangon vulgaris* Fabr. (shrimp), from Boulogne. Probably the course of development is the same as in *Palæmon*, as in the single specimen taken the state of development of the parasite corresponded to the state of development in *Palæmon* at the same date.

*Pathology*.—Everything under *T. octospora* relative to the opacity produced in the host applies equally to *T. giardi*, except that, by reason of the less perfect normal transparency in, and the pronounced tegumentary pigmentation of, *Crangon vulgaris*, the modification is less striking, though it is always sufficiently sharp to permit the recognition of the infected individuals without any difficulty.

*Effects*.—Ehrenbaum<sup>1</sup> noted abnormal individuals of a paler, more opaque color, destitute of the normal greenish tone, apparently considerably enfeebled, dying more rapidly than the normal ones when

<sup>1</sup> Zur Naturgeschichte von *Crangon vulgaris*, Berlin, 1890, pp. 11, 12.

thrown out of the water." The abnormal individuals never included egg-bearing females.

This, Henneguy and Thélohan think, recalls the aspect of Crustacea infected by *Myxosporidia*. They have also never seen egg-bearing females among the infected *Palæmons*. Perhaps we have here, they think, another case of "parasitic castration."

*Infection experiments*.—A *Caradina desmuresti* fed for 71 days with the muscles of an infected *Orangon*, showed, on the most careful examination, no sign whatever of infection.

**33. *Thelohania macrocystis* Gurley, 1893.** Pl. 12, fig. 3.

(Sarcosporidian of *Palæmonetes varians* Garbini,<sup>1</sup> 1891, Rend. Real. Accad. Lincei Roma, VII, Sem. 1, pp. 151, 152 with fig.; myxosporidian of *ibid.*, Thélohan and Henneguy, 1892, Compt. Rend. hebdom. Soc. Biol. Paris, IV, p. 586.)

*Thelohania macrocystis*, Bull. U. S. Fish Com. for 1891, XI, p. 410; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkunde, xv, p. 86.

*Sporophorous vesicle*.—Elongate fusiform. This is the principal character distinguishing this species from *T. octospora*, which has perfectly rounded vesicles.

*Spores*.—Eight in number, pyriform, shell difficultly stainable, coloring only in a 0.5 per cent boiling solution of eosin; spores easily stainable by Gram's method; in the larger posterior end a distinct round "nucleus" more clear and transparent than the surrounding sporoplasm. Together with these forms are others with a thicker and more difficultly stainable shell, within which 8 corpuscles are with difficulty discernible; probably these represent more advanced stages of the same parasite. Garbini failed to find other developmental stages corresponding to those found by Henneguy in *T. octospora*. Inoculation of healthy animals proved a failure.

*Habitat*.—Occurring in great numbers in the muscles of *Palæmonetes varians* (prawn) from the Mincio in the neighborhood of Verona.

*Nature*.—This species has much analogy with *Thelohania octospora*, but presents some noteworthy differences that warrant its specific separation.

## Ordo II. Phænocystes Gurley, 1893.

*Etymology*: φαίνω, I appear; κυστις, capsule.

Bull. U. S. Fish Com. for 1891, XI, pp. 409, 410; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkunde, xv, p. 86.

*Definition*.—*Myxosporidia*, in which the pansporoblast produces few (1 or 2) spores; the latter relatively large, with distinct symmetry and 2 or more capsules;<sup>2</sup> type family, *Myxobolidae*.

<sup>1</sup>First described in Garbini's "Intorno ad un nuovo microorganismo parassita del *Palæmonetes varians* (title only); Atti Real. Accad. Lincei Roma, 1890, VI, p. 526; unpublished.

<sup>2</sup>Except *Myxobolus unicusulatus* and *M. piriformis*. This qualification is omitted by Braun.

## Fam. MYXOBOLIDÆ Gurley, 1893.

(*Myxosporidiæ*<sup>1</sup> Perugia, 1891, Boll. Scientif., Pavia, xiii, p. 23; *Myxobolées* Thélohan, 1892, Bull. Soc. philomat. Paris, iv, pp. 173, 176.)

*Myxobolidæ*, Bull. U. S. Fish Com. for 1891, xi, p. 413; *Myxobolea* [Thél.] Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, p. 739; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Definition*.—*Phenocystes*, whose spores are destitute of antero-posterior, but possess bilateral, symmetry;<sup>2</sup> capsules 2, in 1 group at the anterior end; a bivalve shell, the plane of junction of whose valves is parallel to the longitudinal plane; an iodophile vacuole; type (and only) genus *Myxobolus*.

## IV. MYXOBOLUS Bütschli, 1882.

Etymology not given.

Bronn's Thier-Reich, i, pl. 38, figs. 6-10, and of subsequent authors; *ib.*, Lankester, 1885, Encycl. Britan.; 9 ed., xix, p. 855; *ib.*, Thélohan, 1890, Annal. de Microgr., ii, p. 213; *Myxosporidium*<sup>3</sup> Perugia, 1891, Boll. Scientif., Pavia, xiii, p. 23; *ib.*, Weltner, 1892, Sitzgsber. Gesellsch. Naturf. Freunde Berlin, p. 34; *Myxosporidium*, *ibid.*, p. 35; *Myxobolus et Henneguya*<sup>4</sup> Thélohan, 1892, Bull. Soc. philomat. Paris, iv, pp. 176, 177; *Myxobolus*, Perrier, 1893, Traité de Zool., p. 460; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, pp. 411-13; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

*Definition*.—Characters, those of the family.

*Henneguya* is separated from *Myxobolus* by only 2 characters, viz, (1st) capsules constantly 2, and (2d) the presence of a tail. Inasmuch, however, as all the numerous typical *Myxobolus* species have 2 capsules, and only 2 species are known to deviate in this respect in the direction of capsule-reduction, the typical number of capsules in *Myxobolus* is 2; so that the 2 differential characters in reality reduce to the single one of the presence of a tail. This in itself is not sufficient to warrant a generic separation, especially in view of the entire accord between the tailed and untailed forms in regard to symmetry, similar position of the valves, exactly similar vacuole, nuclei, etc. Besides, it may be noted that it has been several times asserted that tailed and untailed forms occur in the same cyst. Thus Müller,<sup>5</sup> Lieberkühn,<sup>6</sup> and Bütschli<sup>7</sup>

<sup>1</sup> *Myxosporidium* Perugia (synonym for *Myxobolus* Bütschli?) proposed as type of Fam. *Myxosporidiæ* Perugia, by the author in Bull. U. S. Fish Com. for 1891, xi, p. 413.

<sup>2</sup> Except species which have suffered reduction of characters (*Myxobolus unicapsulatus*, *M. piriformis*, *M. inequalis*). Perhaps *M. strongylurus* should be added.

<sup>3</sup> *Myxosporidium merlucii* proposed by the author (Bull. U. S. Fish Com. for 1891 (1893), xi, p. 413) as the type species. The name *Myxosporidium*, having been proposed as a new name for a genus formed by the fusion of several good genera each of which already possessed a name in good standing, must be suppressed.

<sup>4</sup> *Henneguya psorospermica* proposed as the generic type by the author (Bull. U. S. Fish Com. for 1891 (1893), xi, p. 413).

<sup>5</sup> See *Myxobolus* sp. 61, p. 240.

<sup>6</sup> Müller's Archiv., 1854, p. 6; Mém. Cour. et Mém. Sav. Étrang. Acad. Roy. Belg., 1855, xxv, p. 37.

<sup>7</sup> Bronn's Thier-Reich, 1882, i, p. 597. This is probably only an opinion as to the consensus, and not an independent one.

have all asserted this condition. It is, however, almost impossible for me to believe that a tailed species is ever (except of course from breakage, and I have seen many spores deceptively broken) untailed or that an untailed species is ever tailed. I do not recognize as true tails those processes evidently monstrous (as shown by their aspect, their great rarity, their wide divergence from the typical forms, and the lack of transitions thereto) which are very rarely observed in untailed species. Thus I have seen among hundreds of spores of *Myxobolus oblongus* such a form. But that (and also those reported by others belong, I suspect, to the same category) should not be confounded with a true tail. In other words, I believe the presence or the absence of a tail to be a good *specific* character, but not a generic one. Finally, even if the above observations should be admitted to be accurate, might not the conjunction be better explained on the supposition that the 2 forms were in the same *tumor*, but not necessarily (at least until proven) in the same *cyst*, i. e., produced by the same myxosporidium. Although such a close approximation of 2 different species in the same tumor has not been seen, Thélohan is authority for an equally close approximation of 2 different genera in the renal tubules of *Gasterosteus aculeatus* and those of *Pygosteus pungitius*. Finally, in this connection pp. 245, 246 should be consulted. I saw Weltner's results long after writing the above, and perhaps they may demand some modification of it.

*Shell*.—This structure is bivalve throughout the whole of the genus, the valves being superior and inferior.

*Ribbons* ("elastic ribbons" of Balbiani).—These curious and probably abnormal modifications of the ridge are found only in, and are described under, *Myxobolus ellipsoides* (p. 223).

*Tail* (see also pp. 245, 250, 254).—This structure is found only in some species of *Myxobolus*. It was first noted by Müller, who says<sup>1</sup> that it is merely a solid prolongation of the shell substance not containing any extension of the body cavity. This is also, I believe, the view of its structure entertained by all subsequent observers.

Balbiani regards the tail as formed by the coaptation along the median line of his "elastic ribbons" (p. 223). The tail would thus consist of 2 *lateral* halves. This view may be safely rejected, as, if the tail is really composed of two halves, the latter must be *superior* and *inferior*, and not right and left. The latter view of its structure (2 halves, superior and inferior) is taken by Thélohan,<sup>2</sup> who says that the tail is composed of 2 halves (the respective superior and inferior positions of which are necessarily implied, since he says the bifurcation always takes place in the longitudinal plane), whose occasional imperfect coaptation results in the bifurcate condition frequently observed.

Finally, since writing the above, I have been enabled, by the kindness of Prof. Seth E. Meek, to examine *Myxobolus cf. linearis* (p. 253), in

<sup>1</sup> Müller's Archiv., 1841, p. 479.

<sup>2</sup> Annal. de Microgr., 1890, II, p. 206.

which the composition of the tail by the coaptation of a superior and an inferior half is easily demonstrable.

In at least one species, however, this structure of the tail appears not to obtain. In *Myxobolus macrurus* the structure in question seems not to be a shell process at all, but an independent structure with different optical and chemical properties. Although at first inclined to suspect the existence of the two lateral pieces (without the median piece; see p. 250) in the untailed forms, I was unable to detect any trace of them, as iodine failed to separate such a structure. Further, I was unable to prove the constancy of the initial *posterior* divergence of the valves which in *M. macrurus* I suspected to be correlated with the described structure of the tail.

*Sporoplasm*.—Correlated with the typical number and position of the capsules is the characteristic peltate shape assumed by the sporoplasm. The shape and the topographic features of this structure are described in detail under *Myxobolus macrurus* (p. 251). The sporoplasm contains nuclei, an iodophile vacuole, and "granules."

Nuclei (see also "granules" below).—These were first observed by Thélohan. He describes<sup>1</sup> the condition as follows: A series of spores properly stained shows some with 1 nucleus (frequently situated at or near the median cornua) and others with 2, 3, or 4 nuclei, everything pointing to their origin by division from the single one. The subsequent ones appear to migrate at first outward and then backward.

Vacuole (iodophile).—Although visible on some of Müller's figures, Bütschli<sup>2</sup> was the first to direct attention to this structure. He described it as a nucleus, remarking that, though sometimes visible in the fresh state, it became more distinct upon the addition of acetic acid or iodine solution. He failed in his efforts to stain it, a result that he attributed to failure of penetration through the shell of the staining fluid.

In 1889 Thélohan<sup>3</sup> corrected this erroneous interpretation, showing that the structure in question is a vacuole. Little differentiated in the fresh state (on account of similar refrangibility) from the sporoplasm, it becomes evident when the latter is coagulated by alcohol, acetic, nitric, or osmic acids, or by silver nitrate solution (2 per cent). Its chief micro-chemical characteristic is its extreme resistance to nuclear stains, which affect all the surrounding parts.<sup>4</sup> Iodine alone stains it a brownish red, the remainder of the protoplasm taking a pale yellow hue. The iodine reaction exactly resembles that exhibited by glyco-

<sup>1</sup> Annal. de Microgr., 1890, II, p. 210.

<sup>2</sup> Ztschr. f. wiss. Zool., 1881, XXXV, p. 636.

<sup>3</sup> Compt. Rend. Acad. Sci. Paris, CIX, pp. 919-920. For Perugia's confirmation see *M. merlucii*, p. 243.

<sup>4</sup> Bütschli, indeed, states the contrary, but my own results are throughout in accord with those of Thélohan, as are also those of Perugia (Boll. Scientif., Pavia, 1891, XIII, p. 24).



genic matter. The vacuolic contents further resemble the latter in being insoluble in alcohol. Spores kept in this liquid preserve their reaction towards iodine. The vacuolic matter shows a further resemblance to glycogen in its solubility in alkalis. Acids modify it so that after their action it no longer exhibits the iodine reaction. Thélohan was never able to obtain the reduction of the cupro-potassium solution.

Pfeiffer<sup>1</sup> regards it as a nucleus, as does also Weltner.<sup>2</sup>

My own observations are in entire accord with those of M. Thélohan. The structure in question never colors with any staining reagents, nuclear or plasmic. It stains (alcoholic specimens) with iodine, exactly as stated by Thélohan, and is, I think, unquestionably a vacuole.

The vacuole is single, subglobular, usually central or subcentral, differentiated negatively (unstained against a dark ground) by staining reagents, and positively (dark brown against a light ground) by iodine.

Granules ("globules," etc.).—As late as 1884, Balbiani<sup>3</sup> regarded these as latent capsular germs, destined to develop into accessory capsules at the period of reproduction.

These granules appear to be of three kinds:

1. "Globules" present in fresh material. Those situated far forward (usually found at the side of, and apparently connected with, the capsule) were first observed by Bütschli<sup>4</sup> in *Myxobolus mülleri*, and subsequently by Thélohan<sup>5</sup> in *M. oriformis*. I have also seen them in *M. macrurus*. According to Thélohan, these are fatty, as they blacken strongly with osmic acid and dissolve in alcohol.

2. "Granules" distributed irregularly through the plasma are mentioned by Bütschli (*loc. cit.*).

3. The pericornual nuclei. The "granules" forming this series are 2 in number, minute, brilliant, subsymmetrically situated near both the lateral cornua and the posterior extremity of the capsule. These bodies were first noted by Müller.<sup>6</sup> Subsequently (as above mentioned), Balbiani regarded them as capsular germs.

In 1881 Bütschli described at some length the different appearances presented by these bodies in *Myxobolus mülleri* (p. 220).

<sup>1</sup> Die Protozoen als Krankheitserreger, 1891, 2 ed., p. 17.

<sup>2</sup> Sitzungs-Ber. Ges. Naturf. Freunde Berlin, 1892, p. 32.

<sup>3</sup> Compt. Rend. Acad. Sci. Paris, 1863, LVII, p. 160; *Léçons sur les Sporozoaires*, 1884, p. 144. In the latter place he says:

"One remarks in the cavity of the psorosperm other small corpuscles which appear as refringent globules to the number of 3 or 4, symmetrically disposed, often placed at the base of the twin vesicles. I have considered these small globules as vesicles with a filament in a rudimentary state, destined to be developed at the moment of reproduction, for at this moment the psorosperm contains 3 or 4 vesicles with filaments. Bütschli has attacked this manner of view, nevertheless I believe I should maintain it."

<sup>4</sup> Ztschr. f. wiss. Zool., 1881, xxxv, p. 637, pl. 31, fig. 2.

<sup>5</sup> Annal. de Microgr., 1890, II, p. 211, pl. 1, fig. 8.

<sup>6</sup> See p. 240, pl. 28, fig. 6g.

Thélohan<sup>1</sup> was the first to recognize their nuclear nature. He first believed them to belong to the sporoplasm, supposing them to be situated at its 2 antero-external angles (lateral cornua). Subsequently, from a study of capsule development, he<sup>1</sup> regarded the bodies in question as persistent embryonal nuclei, the remnants of such development. He further expressed the belief that these nuclei could in some cases become detached from the capsules and engulfed in the sporoplasm.

Pfeiffer<sup>2</sup> terms them "safranophile corpuscles," but does not comment upon their nature. In *Myxobolus macrurus* I have studied these bodies (which, from their position, may be termed pericornual nuclei) with great care, and with the following results, which apply especially to *M. macrurus*, but equally well to *M. lintoni*:

1. There can be no question whatever that they are nuclei, as they take nuclear stains and show nuclear structure.

2. Their presence or absence and their position (at least in the fully developed spore) appears constant for the same species. As regards constancy of position they contrast strongly with the third and fourth nuclei.

3. The only question is as to their seat. It will be seen above that they have been regarded as belonging to the capsule and also as belonging to the sporoplasm. As is implied by this difference of opinion, their seat is by no means easy of determination, and, after much study, I am as yet uncertain whether they are capsular or sporoplasmic.

Three appearances may sometimes be seen on the same specimen: (a) They appear in one focus-plane almost certainly connected with the infero-lateral cornu; or, (b) they appear almost as certainly attached to the drawn-out posterior end of the capsule; or, (c) they appear disconnected from both and appear to be borne on a broad triangular spur projecting inwards from the shell.

An interpretation which seems possible is that each nucleus is imbedded in the sporoplasm near the tip of the *supero-lateral* cornu, whence it happens that optically its position almost exactly coincides with that of the posterior end of the capsule.

In some species (*Myxobolus cf. linearis*, *M. transovalis*) I failed to find any bodies which on account of the constancy of their position, etc., I could regard as the pericornual nuclei, and this absence appears to be here as definite a specific character as does their presence in *M. macrurus* and *M. lintoni*.

#### 34. *Myxobolus unicapsulatus* Gurley, 1893. Pl. 13, fig. 1.

(Psorosperm of *Labco niloticus* Müller, 1841, Müller's Archiv., p. 487, pl. 16, fig. 5 a-d; *ib.* Robin, 1853, Hist. Nat. d. Végét. Parasites, p. 299, pl. 14, fig. 7.)

*Myxobolus unicapsulatus*, Bull. U. S. Fish Com. for 1891, xi, p. 414; *ib.* of *Labro* [error] *niloticus* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 86.

Cyst and myxosporidium unknown.

<sup>1</sup> Compt. Rend. Acad. Sci. Paris, 1889, cix, pp. 920-1; *ibid.*, 1892, cxv, p. 1097.

<sup>2</sup> Die Protozoen als Krankheitserreger, 1891, 2 ed., p. 7.

*Spore*.—Of the form and size of *Chloromyxum dujardini*. Capsule only 1, situated on one side of the anterior end, obliquely directed.

*Habitat*.—On *Labeo niloticus* from the Nile.

35. *Myxobolus piriformis* Thélohan, 1892. Plate 13, fig. 3 (*pars*), 4 (*pars*)<sup>1</sup>; pl. 18. (Psorosperms of the tench (*pars*) Balbiani, 1883, Journ. de Microgr., VII, pp. 197–198, fig. 66 *b, c, ? d-f*; *ib.* (*pars*) Balbiani, 1884, Leçons sur les Sporozoaires, pp. 125–6, fig. 47 *b, c, ? d-f*; pl. 4, figs. 1, 2, 3A (*pars*)<sup>1</sup>, ? 3B, C; ? *ib.* (*pars*) Pfeiffer, 1890, Die Protozoen als Krankheitserreger, 1 ed., pp. 48, 55, fig. 16; ? *ib.* (*pars*) 1891, 2 ed., p. 132, fig. 56.

*Myxobolus piriformis*, Bull. Soc. philomat. Paris, IV, p. 177; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 414; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 86.

*Synonymy*.—M. Thélohan informs me (letter, 1893) that :

*M. piriformis* has very probably been seen by Remak, although his figures and his descriptions do not prove it absolutely (pl. 5, fig. 5). He does not figure the polar capsules, but his figures almost certainly belong to the species in question.

Fig. 8 represents 2 spores from the kidney<sup>2</sup> of the tench, which I do not know to what species to approximate. The presence of 2 capsules separates them from *M. piriformis*. The form of its spores and the small size of the capsules do not permit of its approximation to any of the forms that I have encountered.

The typical spore of *M. piriformis* contains but 1 polar capsule. As in all species, one can find monstrous spores which inclose 2 capsules, but they have seemed to me very rare. This species is often accompanied, above all in the spleen of the tench, by *M. ellipsoides*. Almost all the spores with 2 capsules, represented by the authors, belong, I believe, to the spores, more or less monstrous, of this last species.

Balbani considered *M. piriformis* a degraded form of *M. ellipsoides*. I have been able to convince myself that this mode of view is not correct. It is a species absolutely distinct and well characterized, as I have been able to determine by numerous observations.

After reading the above, I restudied the synonymy as between this species and *M. brachycystis*, and can not but feel that all of Remak's figures are referable to 1 species, which probably is, as Thélohan thinks and contrary to my former opinion,<sup>3</sup> distinct from his *M. piriformis*. The following are the conclusions at which I have arrived :

(a) Remak's figures are referable to 1 species. His fig. 8 (referred to in the second paragraph of the above quotation) is not from the kidney but from the spleen. There appears to me to be, especially in view of Remak's statements which tend to show that he considered the question carefully, no ground for a separation between these 2 developed spores

<sup>1</sup> The figures in the rows on Balbiani's plate IV, fig. 3, are numbered in order from left to right, in the reproduction of it on pl. 13, fig. 3. The proper specific references of some of the figures of groups 3 and 4, on that plate, are dubious. The following is about all that can be safely said at present :

Indeterminate: Figs. 3 B, C; 4d-f. (either *M. piriformis* or *M. ellipsoides*).

*Myxobolus piriformis*: Figs. 3 A, Nos. 1, 2, 6; 4b, c.

*Myxobolus ellipsoides*: Figs. 3 A, Nos. 3, 4, 5, 7 (the last with some certainty, the rest probably, "abnormal" spores); 4a.

<sup>2</sup> These spores (Remak's fig. 8) are from the spleen.

<sup>3</sup> Bull. U. S. Fish Com. for 1891, XI, p. 409, second footnote, where it is stated that 1 *Myxobolus* species possesses, perhaps inconstantly, a single capsule. At that time I inclined to fuse *M. brachycystis* with *M. piriformis*.

of the spleen and the noncapsulate spores (developing spores; sporoblasts), also from the spleen, shown in Remak's fig. 5. And, finally, between the immature forms of fig. 5 from the spleen and the similarly immature forms from the kidney represented in Remak's fig. 7, specific identity seems almost certain. Another argument which is especially worthy of note is the fact that the spores represented in all 3 figures are *almost exactly the same size*. Remak does not, it is true, state the dimensions in the text, but on the plate he gives the multiplication ratio for the figures, and calculations from careful measurements of them show that all of them agree very closely. I therefore think, with Remak, that they are all one species.

(b) That species is distinct from *M. piriformis*. Among the 3 criteria cited by Thélohan as distinguishing *M. brachycystis* from *M. piriformis*, viz, spore-form, presence of 2 capsules and their small size, especial emphasis should be laid upon the latter, that is upon the small capsular index.

Cyst and myxosporidium unknown.

*Spore*.—Pyriform; closely resembling a pumpkin seed; being flattened-ovoid with a very acutely attenuated anterior extremity. Length, 16 to 18  $\mu$ ; greatest breadth, 7 or 8  $\mu$ .

*Habitat*.—Branchiæ and spleen of *Tinca tinca* L.; kidney of *Misgurnus fossilis*.

**36. *Myxobolus inequalis* Gurley, 1893. Pl. 13, fig. 2.**

(Psorosperms of *Pimelodus blochii* Valenc., Müller, 1841, Müller's Archiv., p. 487, pl. 16, fig. 6a, b; *ib.* Müller, 1843, Rayer's Archiv. de Méd. comp., pl. 9, fig. 6; *ib.* Robin, 1853, Hist. Nat. des Végét. Parasites, p. 299, pl. 14, fig. 8.)

*Myxobolus inequalis*, Bull. U. S. Fish Com. for 1891, XI, p. 414; *Myxobolus inæqualis* [error] of *Pimelodes* [error] *blochii*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkunde, xv, p. 87.

Cyst and myxosporidium unknown.

*Spore*.—Length, 11  $\mu$  (0.0052'''); breadth, 7  $\mu$  (0.0033'''); capsules 2, of unequal size.

*Habitat*.—On *Pimelodus clarias* Bloch (= *Silurus clarias* Valenc.) from Guiana and Surinam.

**37. *Myxobolus brachycystis* sp. nov. Pl. 14, figs. 1-3.**

(Psorosperms of *Tinca chrysitis*, Remak, 1852, Müller's Archiv., pp. 144-146, pl. 5, figs. 5, 7, 8.)

Compare carefully p. 211. Remak compares it (by reference to Müller's figures) to *Chloromyxum dujardini*.

*Spore formation*.—Pansporoblast: Oval vesicles usually situated on the walls of the blood vessels of the kidney or spleen; either in connection with, or separate from, the pigment follicles; pansporoblast always monosporogenetic. In the developing spores Remak not infrequently missed the capsules, but comparison with developed forms which occurred in other cases left no doubt as to their nature.

*Spore*.—Pyriform, long drawn out.

*Habitat*.—Remak gives this as the pigment follicles of the spleen and

of the kidney of *Tinca tinca* L. (tench). He further asserts that the same form is found on the branchiæ, but as he does not figure any spores from the last seat it may perhaps be a question whether the branchiæ yield the present species in addition to *M. piriformis*.

In the kidney a 3-chambered pigment cyst was seen  $27\ \mu$  ( $\frac{1}{80}$ ''') long, the end compartments of which were occupied by pigment and the central one by a pyriform spore.<sup>1</sup> The pigment-follicles of the spleen almost always contain untailed psorosperms in considerable numbers, lying without order between the pigment-holding cells. The pigment follicles of the kidneys always contain the same species as that found in the spleen and upon the gills (Remak).

**38. Myxobolus ? sp. incert.** Pl. 14, fig. 4.

Psorosperms of *Cyprinus tinca*, Lieberkühn, 1854, Müller's Archiv., pp. 6, 24, 353, pl. 2, figs. 21-27.

Lieberkühn's description is substantially as follows:

Cyst imbedded in cornea immediately under the inner surface. Upon slight pressure very many spores, partly with and partly without tail-like appendages, and whose shell was no longer smooth but wrinkled, and whose capsules were no longer together but occupied unusual positions, were seen. Individual shells contained only 1, and others no capsule. A number of free "nuclei" which had preserved the club-shape of those within the spore also were seen. Finally, very small diaphanous, nongranular, amœbiform corpuscles occurred, which plainly, though slowly, moved with blunt or sharp processes.

*Habitat*.—Encysted in cornea of *Tinca tinca* L. (tench).

Concerning these figures, Thélohan (letter to author, 1893) says that they are not to be approximated to *M. piriformis*. Lieberkühn's fig. 21 would, he says, rather suggest *Chloromyxum dujardini*.

**39. Myxobolus ? mugilis** Perugia, 1891. Pl. 14, figs. 5, 6.

*Myxosporidium mugilis* Perugia, Boll. Scientif., Pavia, XIII, pp. 23-24, plate, figs. 7, 8; *ib.*, Weltner, 1892, Sitzungsber. Gesellsch. Naturf. Freunde Berlin, p. 35.

*Myxobolus mugilis* Thélohan, 1892, Bull. Soc. philomat. Paris, IV, p. 166; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 414; *ib.* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Cyst membrane*.—Having removed with care one of the cysts from the branchiæ of *M. capito*, Perugia observed it to consist of 3 (others contain 2) separated myxosporidia surrounded by a common investing membrane evidently derived from the branchial lamella, which latter at no point showed any solution of its continuity. From this he concluded that the cyst is a production of the host. Some cysts contain 2 or 3 myxosporidia filled with spores, and with a residue of a very few granulations of protoplasm.

*Myxosporidium* not described.

*Spore*.—Free; "without a proper membrane"<sup>2</sup>; length,  $7\ \mu$ .

*Habitat*.—Encysted in the branchial lamellæ of *Mugil auratus* and of *M. capito* (gray mullets). Rare; found only twice in 300 *Mugils*.

<sup>1</sup> Remak here erroneously refers to his fig. 5a instead of fig. 7A.

<sup>2</sup> From other similar expressions by the same author I interpret this to mean: "No pansporoblast membrane."

Relative to its generic relations Perugia says:

This form might be referred to the genus *Myxobolus*, from which it seems to me to differ only by a little. The different hosts and the form of the spores only might cause it to be regarded as a distinct species.

**40 *Myxobolus* sp. incert.** Pl. 14, fig. 7.

(Psorosperm of *Nais proboscidea*, Lieberkühn in Bütschli, 1882, Bronn's Thier-Reich, I, p. 590, pl. 38, fig. 23; *ib.*, Thélohan, 1890, Annal. de Microgr., II, p. 193; *ib.* Pfeiffer, 1890, Virchow's Archiv. f. pathol. Anat. u. Physiol., CXXII, p. 557; *ib.* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739.)

No description. Its symmetry shows it to be a *Myxobolus*. Observed by Lieberkühn, and communicated by him to Bütschli; published only by the latter.<sup>1</sup>

*Habitat.*—*Nais proboscidea* (a worm).

**41 *Myxobolus* sp. incert.** Pl. 15, figs. 1-6.

Psorosperms of *Esox lucius*, Lieberkühn, 1855, Mém. Cour. et Mém. Sav. Étrang. Acad. Roy. Belg., XXVI, p. 37, pl. 10, figs. 10-12, pl. 11, figs. 1-4; ? *ib.* Bütschli 1882, Bronn's Thier-Reich, I, pl. 38, fig. 11.

*Cyst.*—Size 8 mm. (0.31 inch) by 4.25 mm. (0.17 inch); contents "granular matter" alone, spores alone, or both "granular matter" and spores, in variable proportion.

Myxosporidium unknown.

*Spore.*—Oval or circular, tailed or untailed; the 2 kinds often mixed without order in the same cyst.

*Habitat.*—Cysts of branchiæ of *Lucius lucius* L. (pike).

It is hard to know what to do with this form. In spite of his assertion that tailed and untailed forms occur in the same cyst, Lieberkühn appears to figure only untailed forms. In view of this, and provisionally until some other observer shall confirm this observation, I prefer to recognize this as a "form" distinct from the tailed one having approximately the same habitat. (See also p. 256.)

**42 *Myxobolus oviformis*** Thélohan, 1892. Pl. 14, fig. 8.

("Myxosporidian spore (*M. mülleri* Bütschli?)" of *Cyprinus carpio* and of *Gobio fluviatilis*,<sup>2</sup> Thélohan, 1890, Annal. de Microgr., II, pp. 200, 204, 209, 210, 211, 213, pl. 1, figs. 8-11; spore of *C. carpio*, Thélohan, 1890, Compt. Rend. Acad. Sci. Paris, CIX, p. 921).

*Myxobolus oviformis* Thélohan, Bull. Soc. philomat. Paris, IV, p. 177; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 414; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

Cyst and myxosporidium not mentioned.

*Spore.*—Flattened-ovoid, with notably attenuate anterior extremity; length, 10 to 12  $\mu$ ; breadth, 8 $\mu$ ; capsules relatively large (6 $\mu$ ); nuclei *ad plur.*, 3; vacuole, present.

<sup>1</sup> Braun's language is slightly ambiguous: "Eine ältere Notiz, von Lieberkühn, erwähnt" the occurrence of *Myxosporidia* in invertebrates.

<sup>2</sup> An ambiguous expression of Lieberkühn's (Bull. Acad. Roy. Belg., 1854, XXI, pt. 2, pp. 22-23) may refer to an observation of a species upon the branchiæ of this fish.

*Habitat*.—Common on fins (where the spores exist in great numbers in the subcutaneous tissue) of *Gobio gobio* L. (gudgeon); branchiæ of same fish, of *Cyprinus carpio* L. (carp), and of *Alburnus alburnus* L.

43. *Myxobolus* ? cf. *oviformis*.

Psorosperms of *Cyprinus carpio*, Balbiani, 1883, Journ. de Microgr., vii, pp. 199-201; *ib.*, Balbiani, 1884, Leçons sur les Sporozoaires, pp. 128, 130, 131.

Cyst and myxosporidium not mentioned.

*Spore*.—Length 18  $\mu$ ; breadth 12  $\mu$ .

*Habitat*.—On *Cyprinus carpio* L. (carp).

The dimensions differ so markedly from those of *M. oviformis* that on the present evidence I have not felt justified in fusing the 2 forms. It is, however, worthy of note that the ratio between the dimensions is the same as that in *M. oviformis*, and also that "18" may not impossibly be an error for 8. M. Thélohan writes that he has never found in the carp spores measuring 18 by 12  $\mu$ , and suggests that these dimensions may be an error.

44. *Myxobolus* sp. incert. Pl. 15, fig. 7.

Cyprinus brama, "psoro- sperms," etc., of—	Gobio fluviatilis [error] myxosporidian spore of—	Date.	Authority; reference.
Cf.	.....	1841	Müller, Müller's Archiv., pp. 491-2.
×	.....	1854	Lieberkühn, Müller's Archiv., p. 368, pl. 14, figs. 7, 8.
×	.....	1879	Leuckart, Die Parasiten des Menschen, p. 248, fig. 99b.
	×	1882	Bütschli, Bronn's Thier-Reich, I, p. 600.
	×	1882	Lieberkühn in Bütschli, Bronn's Thier-Reich, I, pl. 38, fig. 18a-c.
×	.....	1886	Leuckart, The Parasites of Man, 2 ed., p. 197, fig. 99B.
×	.....	1887	Koch, Encyklop. d. gesamt. Thierheilkde u. Thierzucht, IV, p. 94, fig. 668, 2, 3.

Bütschli's reference to *Gobio fluviatilis* is certainly an error. His figs. 18b and 18c (loaned him by Lieberkühn) are respectively copies of Lieberkühn's figs. 7 and 8. That they are not merely independent figures of specifically identical material can be seen from the identity of the figure of the ever-varying amœboid (fig. 8, Lieberkühn; fig. 18c, Bütschli; see pl. 15, fig. 7c). The question is, moreover, additionally settled by Prof. Bütschli's statement that—

Concerning the subsequent fate of the spore, only two observers, Lieberkühn and Balbiani, have so far expressed opinions. They agree that the spore-shell finally separates, the protoplasmic contents emerging as a small active amœboid body (18b, c).

Thus the 2 figures in question were copied. Further, Lieberkühn mentions a "psorosperm" from the body cavity of *Gobio fluviatilis* (see p. 243), and describes in detail his observations in that form upon the separation of the valves and the exit of the amœboid posterior mass. He makes no mention, however, of any forms upon the branchiæ of *Gobio fluviatilis*. The fact that Bütschli cites its habitat as the branchiæ, with his statement that in this matter he is quoting, estab-

lishes the conclusion that his reference to *Gobio fluvialis* was due to an erroneous correlation between Lieberkühn's text and Lieberkühn's figures. Finally, Bütschli's fig. 18a appears to be the transverse view of 18b.

Concerning the relation between this form and *M. sp.* 45, M. Thélohan (letter to author, 1893) says:

It is impossible to say whether this figure should be approximated to my *Myxobolus* of the bream.

No description.

*Habitat*.—Branchiæ of *Abramis brama* L. (bream).

**45. *Myxobolus sp. incert.***

*Myxobolus* of bream, Thélohan, 1892, Bull. Soc. philomat. Paris, iv, p. 178.

Cyst and myxosporidium not mentioned.

*Spore*.—Length, 8  $\mu$ ; breadth, 6 to 7  $\mu$ .

*Habitat*.—Branchiæ of *Abramis brama* (bream).

*Remarks*.—Differs from *M. mülleri* only in the smaller size of the spores. See also remarks on the preceding species.

**46. *Myxobolus mülleri* Bütschli, 1882. Pls. 16, 17.**

(Myxosporidian spores of *Squalius cephalus*, of *Barbus fluviatilis*, and of other fresh-water Cyprinoids, Bütschli, 1881, Ztschr. f. wiss. Zool., xxxv, p. 630, footnote, pp. 630-8, 646-8, pl. 31, figs. 1-24.)

*Myxobolus mülleri*, Bronn's Thier-Reich, i, pp. 595-7, pl. 38, figs. 6-10; *ib.* Lankester, 1885, Encycl. Britan., 9 ed., xix, p. 855, fig. xvii, 40, 41; *ib.*, Leunis, 1886, Synopsis d. Thierkde, ii, pp. 1137-8, figs. 1118-9; *ib.*, Thélohan, 1892, Bull. Soc. philomat. Paris, iv, pp. 166, 167, 178; *ib.*, Gurley, 1893, Bull. U. S. Fish. Com. for 1891, xi, p. 414; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, p. 739; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Synonymy*.—Bütschli (1881) says the *Myxosporidia* investigated by him came principally from the Cyprinoids, but that he could not give the species of host exactly, as he investigated large numbers of excised branchiæ. In part, however, these latter were derived from *Squalius cephalus* and from *Barbus fluviatilis*. He further states that he was unable to recognize any specific distinctions between the spores of the series he examined. Bütschli's type figures of 1882 are copies of his figures of 1881. Parenthetically, also Lankester's and Leunis's are copies of these. Of those who have studied the pathogenic muscle-form of *Barbus barbus* (= *fluviatilis*), all admit its close similarity to, and some assert its identity with, *M. mülleri* (see p. 225). Further, Pfeiffer states that in the Rhine basin, in which the epidemic produced by the muscle-form is very extensive, the branchiæ are free from *Myxosporidia*, a nonassociation that would seem to favor the idea of specific distinctness. So far, then, no direct comparison has been made between the spores inhabiting the branchiæ of *B. barbus* and those inhabiting the muscles of the same fish. In the meantime it is probable that *Leuciscus (squalius) cephalus* L. should be regarded as, so to speak, the type host of *M. mülleri*.



*Cyst.*<sup>1</sup>—Exclusively confined to the branchial lamellæ, appearing by reflected light as white pustules, usually elongate-oval, 2 to 3 mm. long; with greater development distending the flat branchial lamellæ. On closer examination of the freshest possible branchiæ, the cysts are seen to be neither extra-, nor intra-, but sub-epithelial, the blood vessels of the mucosa running over their surfaces. Their seat is thus the submucous connective tissue layer which immediately surrounds the supporting central cartilaginous rod of the lamella, and which underlies each and separates both of the layers of mucous membrane, which latter form the opposite faces of the lamella and in which run, superficially, the afferent and efferent blood vessels and the capillaries of the mucosa. One can easily convince himself of this situation of the myxosporidium by external observation. One then remarks that the transverse-running capillaries superficially girdle the myxosporidium. A transverse section through the mass thus shows the supporting central cartilaginous rod girdled by the myxosporidium, and the latter in its turn surrounded by the vascular layer of the mucosa. If the myxosporidium attain a greater growth, it naturally distends the lamellæ more and more, and, since the transverse capillaries girdle the myxosporidium ring-wise and oppose an obstacle to its expansion, the latter structure bulges out, sac-like, in the intervals between them, its whole outline being thus multilobate. From some further observations on very large myxosporidia, Bütschli believes that finally, through the continued growth of the myxosporidium, the restraining capillaries become ruptured, which explains the blood extravasations observed by him in the superficial portions of large myxosporidia, the girdling capillaries in these cases being absent.

**Membrane:** By careful manipulation the myxosporidium can sometimes be removed intact from its seat in the branchiæ. In both of the two successful instances, Bütschli observed a distinct membrane which possessed special interest in differing from the type usual among the unicellular organisms and particularly from that found in the Gregarines. It is of a plasmatic nature, being composed of clear, very finely granular protoplasm, in which numerous small nuclei are imbedded. Neither acetic acid nor staining reactions show any evidence of cell outlines. The finely granular nuclei possess a distinct dark membrane, show a somewhat irregular outline, and stain intensely with alum carmine. It is difficult to determine with certainty whether this membrane is a production of the myxosporidium or of the tissues of the host. As opposing the former view (a view which, however, Bütschli considers as in no wise excluded) is the fact that the nuclei of the membrane are somewhat larger than those found in the endoplasm.

<sup>1</sup> The description is Bütschli's. He calls it the myxosporidium, but it appears from his description to be the cyst (which, however, is probably only a later stage of growth of the imbedded myxosporidium). Pfeiffer erroneously states that these observations were made upon *Perca fluviatilis* (Die Protozoen als Krankheitserreger, 2 ed., 1891, p. 130).

*Myxosporidium*.—Myxosporidium usually showing no clear differentiation of ectoplasm and endoplasm except in thin sections, where certain portions exhibit very plainly a tolerably thick, granule-free exterior zone, possessing a great interest on account of its very distinct fine radiate striation. Endoplasm thickly studded with very small but distinct nuclei which in thin sections are, even in the fresh state, rather plainly visible as faint roundish corpuscles, in which dilute acetic acid differentiates a dark somewhat granulated membrane, a small dark nucleolus, and, sometimes quite clearly, fine nuclear threads radiating from the nucleolus to the membrane. This structure, together with their intense affinity for stains, permits no doubt as to their nuclear nature.

*Spore formation*.<sup>1</sup>—This species never shows a paired spore-development, or a development within a pansporoblast (?; see below), the spores being directly imbedded in the endoplasm. These spores, however, show indications of a similarity in their development to the other *Myxosporidia* in their origin from a trisegmented ("trinucleate") plasma-globule, 2 of whose segments develop the capsules and the third the sporoplasm.

*Development of spore*.<sup>2</sup>—In the myxosporidium, inclosed in a delicate membrane, a number of mature spores are seen, many things pointing to their origin from the protoplasm. They always contain 3 pale, almost spherical, but somewhat angular bodies. The membrane frequently shows an excavation and an opening at one end. At this end the 2 protocysts are situated, the protosporoplasm being remote therefrom. Further observation shows the protosporoplasm to develop into the sporoplasm of the mature spore and the two protocysts to give origin to the capsules. The latter structures develop within the protocysts, the filament appearing first in the extruded condition, apparently forming a prolongation of the capsular wall.

Subsequently, in the light of his observations on the development of *Myxidium lieberkühnii*, Bütschli inclined to interpret thus: That the 3 spheres (viz, the 2 protocysts and the protosporoplasm) represent not plasma-spheres but nuclei, the latter being, on this supposition, imbedded in a plasma mass which he had failed to see, probably on account of strong swelling and great transparency.

The observations of Balbiani and of Thélohan, however, render it almost certain that Bütschli's observations were accurate and that his subsequent interpretation was erroneous (see also pp. 82, 223). Upon this view the present species would seem to develop pansporoblasts, each with a single spore.

*Spore*.—Lenticular-oval, anterior end sharpened, showing quite plainly a shallow funnel-shaped depression; posterior end rounded off; dimensions 10 to 12  $\mu$  by 9 to 11  $\mu$ . On vertical view, contour rather variable,

<sup>1</sup> Bütschli, 1882, Bronn's Thier-Reich, I, p. 597.

<sup>2</sup> The description is Bütschli's (Ztschr. f. wiss. Zool., 1881, xxxv, pp. 646-8).

often almost circular, anterior end only slightly attenuated, border of suture exhibiting folds or crimpings varying in number from 7 to 9.

Shell: Substance dark and somewhat glittering, possessing a marked resistance to chemical reagents; warmed with concentrated sulphuric acid the valves fall apart; stronger heating effects their complete destruction. Valves 2, superior and inferior, with a tolerably thick ridge or welt along the border (line of junction), visible very plainly as a ridge on transverse view.

Capsule: Wall tolerably thick, glittering, inclosing a cavity occupied by the coiled filament which appears paler than the wall; showing, with the normal extrusion of the filaments, a very noticeable diminution of volume, whence the conclusion that (as with the thread-cells proper) such extrusion is the result of the pressure of the stretched elastic capsular walls. The capsules are destroyed by gently warming with concentrated sulphuric acid. Filaments extruded under the influence of potassium hydrate solution, glycerin, and especially concentrated sulphuric acid; also by mechanical pressure. The extrusion produced by the last means is frequently abnormal and very irregular, the filament being ejected in a more or less spiral form, or only incompletely, or sometimes through a rupture in the capsular wall, either into the shell cavity, or through the shell, or, in the last case, more probably between the (by the pressure) partially loosened valves. Bütschli adds a few interesting remarks to the effect that the capsules, so constant in the *Myxosporidia*, doubtless have some important and yet to be discovered function.

Sporoplasm: Mostly very delicate, cloudy, granulated, nearly filling the posterior portion of the shell cavity, projecting forward in the median line and on the outer side of the capsules; this projection could not be traced all the way around the capsules. Containing a variable number of granules. Vacuole,<sup>1</sup> frequently quite plainly visible even in the fresh state as a circular or oval clear spot. It becomes more prominent, however, after the addition of dilute acetic acid or iodine solution and then shows a dark, somewhat granulated membrane and a number of rather pale granules strewn through the contents, resisting all stains,<sup>2</sup> according to Bütschli sometimes invisible, a result that he attributes to great condensation of the protoplasm. Some spores appeared to possess 2 vacuoles, but upon this point Bütschli was not certain.

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<sup>1</sup>This is Bütschli's description of his "nucleus."

<sup>2</sup>A circumstance explained (but erroneously) by Bütschli as being due to a failure of the stain to permeate the shell. He says the nonstaining can not be taken as a contraindication of the nuclear nature of the structure in question, as the protoplasm also resists the stain. From my own experience I should say that would depend on the kind of stain used, plasmatic stains generally being, nuclear stains generally not being, retained.

"Granules."—Bütschli summarizes his results thus:

There are very constantly found in the protoplasm 2, or sometimes more, strongly refractile glittering granules of a roundish form. They are usually, though by no means always, situated tolerably symmetrically, just at the posterior ends of the polar capsules. No decided regularity obtains either as regards the number or position of the granules, as they are sometimes placed farther forward between the capsules, and sometimes are strewn entirely irregularly through the plasma.

I have also observed, with longer preservation of the spore in water, an appearance which was not clearly intelligible, but which I will briefly describe. In spores so preserved one sees after some time nothing more of the 2 dark granules usually present, but on the other hand one sees on each polar capsule posteriorly a dark punctule which occupies nearly the same position as the above-mentioned granule. It gives the impression as though the dark granule had fused with the capsular membrane and had developed into the punctule. I must, however, regard the interpretation mentioned as a mere conjecture.

*Effects*.—Invades the connective tissue and ovules of *Phoxinus phoxinus* (Thélohan, 1892).

*Habitat*.—Branchiæ of various cyprinoids, particularly *Leuciscus* (*Squalius*) *cephalus* L.; *Barbus barbus* L. (barbel), both *vide* Bütschli. Fins of *L. cephalus*: kidney and ovary of *Phoxinus phoxinus* L., and on *Crenilabrus melops* at Roscoff (Thélohan).

**47. Myxobolus? sp. incert.**

Psorosperm (second species) of *Platystoma fasciatum* Müller, 1841, Müller's Archiv., p. 489.

Cyst and myxosporidium unknown.

*Spore*.—Oval, untailed; size equals that of *M. sp.* 61.

*Habitat*.—On branchial arches (especially at their angles where the mucous membrane is soft) of *Pseudoplatystoma fasciatum*.

**48. Myxobolus bicostatus** Gurley, 1893. Pl. 19, fig. 1.

(Myxosporidian spore of *Tinca vulgaris*, Lieberkühn in Bütschli, 1882, Bronn's Thier-Reich, I, pl. 38, fig. 19.)

*Myxobolus bicostatus*, Bull. U. S. Fish Com. for 1891, XI, p. 414; *ib.* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

No description.

*Habitat*.—Branchiæ of *Tinca tinca* L. (= *vulgaris*), tench.

This species is distinguished from *M. ellipsoides* by its larger capsular index (0.50 as against 0.33 in *M. ellipsoides*) and by the 2 oblique ribs on the shell.

49. *Myxobolus ellipsoides*, Thélohan, 1892. Pl. 13, figs. 3, 4<sup>1</sup>; pls. 18, 20; pl. 19, figs. 2-8; pl. 21, figs. 1, 3*d*, 5, (? 2, 3*a-c*, *e*; ?? 4; ? pl. 22, figs. 1-3).

Tench "psoro- sperms" of, spores of, etc.	Pike, [Error] "psoro- sperms" of.	ellip- soides.	Date.	Authority; reference.
×	-----	-----	1863	Balbani, Compt. Rend. Acad. Sci. Paris, LVII, p. 160.
×	-----	-----	1864	Balbani, Gaz. Méd., Paris, XIX, p. 146.
×	-----	-----	1874	Moreau, Compt. Rend. Assoc. franc. Avanc. Sci., 2 <sup>e</sup> (Lyons) Sess., p. 814.
×	-----	-----	1883	Balbani, Journ. de Microgr., VII, pp. 199, 201-2, 272-4, 276-9, figs. 40, 61-3, 65 <i>a</i> (see p. 211).
×	-----	-----	1884	Balbani, Leçons sur les Sporozoaires, pp. 127-8, 130, 137-40, 142-6, 148, figs. 36, 42-44, 46 <i>a</i> ; pl. 3, fig. 9; pl. 4, figs. 1-3 ( <i>vars</i> ; see p. 211).
×	-----	-----	1886	Railliet, Elém. Zool. Méd. et Agric. Paris, pp. 167-8, fig. 72.
×	-----	-----	1887	Pfeiffer, Ztschr. f. Hygiene, III, p. 475, fig. 2 <i>e, f, g</i> .
×	×	-----	1888	Pfeiffer, Ztschr. f. Hygiene, IV, pp. 409, 417-20, fig. 15 <i>a-c</i> .
×	-----	-----	1889	Henuguy, Dict. Encyclop. d. Sci. Méd., p. 775, figs. 2 <i>a-h</i> .
×	-----	-----	1889	Thélohan, Compt. Rend. Acad. Sci. Paris, CIX, pp. 920-1.
×	-----	-----	1890	Thélohan, Annal. de Microgr., II, pp. 198, 200-4, 207, 209, 210, pl. 1, figs. 2, 3, 12-16.
×	-----	-----	1890	Thélohan, Compt. Rend. Acad. Sci. Paris, CXI, p. 695.
×	-----	-----	1890	Pfeiffer, Arch. f. pathol. Anat. u. Physiol., CXXII, pp. 558-9, 563.
×	-----	-----	1890	Pfeiffer, Die Protozoen als Krankheitserreger, 1 ed., pp. 44, 47, 48, figs. 14, 16 (part; all ?).
×	-----	-----	1891	Pfeiffer, Die Protozoen als Krankheitserreger, 2 ed., pp. 130, 133-4, figs. 54, 56 (part; all ?).
-----	-----	Myxobolus.	1892	Thélohan, Bull. Soc. philomat. Paris, IV, p. 177.
-----	-----	do	1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, p. 414.
-----	-----	do	1893	Braun, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 739.
-----	-----	do	1894	Braun, Centralbl. f. Bakt. u. Parasitenkde, XVI, p. 87.

*Synonymy*.—The number of known forms habitant on *Tineá tineá* is large and their relations *inter se* are dubious. By the separation of *M. piriformis*, Thélohan has made a decided advance in the direction of clearness. By its lanceolate shape, single capsule, and large capsular index it is distinguished clearly from *M. ellipsoides* and from *M. brachycystis*. It is probable that some of Pfeiffer's degenerated forms should receive a somewhat similar interpretation. His figures are, however, such that in the absence of more definite statements they can hardly be placed. One of them (pl. 21, fig. 3*d*) would seem to belong to this species. The others are entirely indeterminate.

*Cyst*.—Thélohan (1890, p. 203) saw cysts enlarge, become submucous, distending the mucous membrane, which subsequently ruptured, permitting the cyst to shell out and fall into the water, where it burst exactly as with the subcutaneous cysts of *Gasterosteus aculeatus*. Cysts are found in the comparatively exposed parts, e. g., the subcutaneous and intermuscular connective tissue and in the subepithelial tissue of the branchiæ, being absent in the internal organs (air-bladder, etc.).

*Myxosporidium*.<sup>2</sup>—(a) In the air bladder: Two forms occur in the air-bladder of the tench; the first very similar to that found in the

<sup>1</sup> See p. 211, footnote 1, and the explanations of the plates.

<sup>2</sup> Thélohan, Annal. de Microgr., 1890, II, pp. 201-2.

urinary bladder of *Lucius lucius*, consisting of small free masses lining the internal surface of the organ, the second consisting of drawn-out, chain-like masses in the midst of the tissues of the organ. The second he believes to be merely a more advanced stage of the first. When the parasite is only slightly developed its presence is recognizable only by small opaque streaks in the otherwise transparent bladder, on opening which the myxosporidium is found upon its internal surface. In other cases small white prominences are found, presenting a transition between the large mammillated masses described by Balbiani, and which can attain 10 mm. in thickness. Sections show the myxosporidium intimately united to the epithelium. The latter soon becomes broken up and the plasmic chains insinuate themselves between the fibers of the connective tissue.

By serial sections one can follow progressively the march of the parasite into the tissues. These last allow of separation and stretching of the fasciæ, such change being progressive and slow. Soon, however, under the continuous pressure produced by the growth of the invading mass, the fibers arrive at the limit of extensibility and finally rupture. Thus are formed irregular spaces, in the middle of which one finds the débris of the tissue of the organ, surrounded by the myxosporidia. During this time spores are formed. They finally almost entirely replace the protoplasm. In other parts of the same mass earlier and intermediate stages can be seen. In the air bladder, as in the kidney, the distinction between the ectoplasm and endoplasm is little evident and, beyond the fact of the absence of nuclei from the ectoplasm, it is difficult to find characters to separate these layers.

(b) Of the external surface, Balbiani<sup>1</sup> gives, as the results of his investigations, the following account of the development:

Of all freshwater fishes the tench is most frequently affected with *Myxosporidia* and at all seasons. This, together with the transparency of the fins of the young, renders it especially favorable for investigation. Balbiani frequently observed upon the fins, mingled with developed psorosperms, small amœboid bodies of very variable size. These move like the most agile amœbæ (e. g., *A. diffluens*), 9 changes of form occurring in less than 15 minutes; temperature had great influence, heat accelerating, cold retarding. The pseudopodia were large and obtuse, the mass appearing lobed, as in *A. diffluens*. Unless obscured by fat globules (numerous in the later stages), the nucleus is plainly visible, particularly at the time of the exit of the mass from the spore. It is the nucleus of which Bütschli has proven the existence in the interior of the psorosperm (cf. p. 208). There is no contractile vacuole, and from this point of view these bodies differ from the ordinary amœbæ.

While thus wandering over the fins, the small amœboid bodies absorb nutriment, grow, show more or fewer fatty globules, tend to take a rounded oval, or sometimes irregular form with expansions and lobes, and to surround themselves with a thin envelope easily visible in water. As the water penetrates the fin tissue, the amœboid movements become more and more slow and finally cease. Independently of its thin proper membrane, the small mass is encysted in the same manner as other foreign bodies, by the connective tissue of the host.

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<sup>1</sup> Journ. de Microgr., 1883, VII, pp. 272-4.

*Spore formation.*—With the growth the number of nuclei increases by successive divisions<sup>1</sup> (many of which were seen to occur). Subsequently each nucleus condenses around it some of the myxoplasm, thus forming the pansporoblasts. These grow, become elliptic, and the rudiments of the capsules appear in them, at first as very pale, then as brilliant bodies. The mode of their development was not entirely satisfactorily ascertained. They usually develop 2 in each pansporoblast, some of these sporoblasts containing 3 granular globules, 2 small and 1 large, which probably develop respectively into the capsules and the sporoplasm. Also incompletely developed spores were seen inclosing elements believed to be capsules in process of development. These were: (1) Two spherical vesicles containing each a small central globule placed in the substance of the spore remote from the poles. (2) Two small similar vesicles placed one beside the other at one pole. (3) Two pyriform vesicles with a small central globule, sometimes remote from each other, sometimes approximated to each other and situated at one extremity of the spore. These vesicles were no doubt the small organs with spiral filaments. Their origin could not be clearly determined.

*Spore.*—Flattened-ellipsoid, rather elongate, the two ends similar; length 12 to 15  $\mu$ ; breadth 9 to 11  $\mu$ ; length of capsules 4  $\mu$ ; nuclei of capsulogenous membrane persisting to maturity of spore; vacuole present; nuclei originating by continued division from a primitive one, not more than 4; when of this number, 2 are situated before and 2 behind the vacuole (Thélohan, pp. 209–210).

Degenerate forms [of this species ?] from the gall bladder may have 3 capsules or none, and the bivalve character of the shell may be absent (Pfeiffer).

Ribbons: Balbiani<sup>2</sup> has made some curious but dubious observations, arriving at conclusions which by no means accord with the general consensus of opinion. He describes an elastic, ribbon-like process (the *ribbon*) as existing along the border of each valve of the shell, stating that at the time of maturity of the spore (the only period at which such ribbons are visible, as at other periods they are closely appressed to the valves) they become unrolled and recurved, such action resulting in the splitting apart of the valves and the consequent release of the amœboid sporoplasm. The ribbons divide at their distal extremity into 2 or 3 *ribbonettes*. These elastic structures he regards as comparable to the cruciform elastic filaments (*elaters*) of the *Equisetum* spore, remarking that in the *Myxosporidia* they serve a different function, their action here being valve-separation and not spore-dispersal. He further says that these elastic ribbons have another function, viz, to maintain contact of 2 spores during what he regards as a state of

<sup>1</sup>From Balbiani's language it is plain that he did not recognize the vacuolic nature of Bütschli's "nucleus." Still he must have seen nuclei (and not vacuoles) in the later myxosporidium stages, as he states that he repeatedly observed them to divide. Probably Thélohan's observation of karyokinetic division (Compt. Rend. Acad. Sci. Paris, 1890, cxi, p. 693) was upon *M. ellipsoïdes*, though it is not distinctly so stated. Among other figures he saw a spindle with an absolutely typical equatorial plate.

<sup>2</sup>Journ. de Microgr., 1883, vii, pp. 276–7: *Léçons sur les Sporozoaires*, 1884, pp. 142–4.

conjugation. And still further, in some individuals the filaments instead of lying along the borders of the valves, extend themselves in the direction of the axis of the body, and, reuniting themselves for a variable distance, constitute the simple or double caudal prolongation that Müller and other observers describe as a specific character of certain psorosperms. (See also p. 207.)

Concerning these, Bütschli<sup>1</sup> states that he could find no evidence whatever of the existence of such ribbons, either in the whole spore or in the separated valves. He seems to think that such ribbons are an illusion due to an abnormal extrusion of the capsular filaments.

Thélohan's observations seem to throw some light upon this discrepancy. This observer<sup>2</sup> says that he has never seen them except in the present species. They are frequently absent, yet the spores split open perfectly. Having found all possible transitions between the ribboned spores and spores evidently monstrous and abnormal, he regards the ribbons as structures, accidental rather than fundamental and necessary to the development of the spore.

*Habitat.*—Thélohan gives this as the branchiæ, air bladder, liver, intestine, and spleen (last *vide* letter to author, 1893) of *Tinca tinca* L. (tench). Balbiani says the *Myxosporidia* are always confined to the short anterior portion of the air bladder.

Speaking collectively of a poorly delineated and very probably multi-specific group of forms, Pfeiffer says that perfectly developed forms occur on the branchiæ and in the air bladder, this stage of development being possibly connected with an abundance of oxygen. In the gall bladder incompletely developed forms occur, with 3, 1, or no capsules; also entirely undeveloped forms, destitute of a bivalve shell, comparable to the *Microsporidia* or to the pseudo-navicellæ found in *Lumbricus*. Transition forms to the *Coccidia* also occur. Possibly (from Pfeiffer's figure) *M. ellipsoides* may also occur in the air bladder or gall bladder.

*Effects.*—The *Myxosporidia* do not confine themselves to existing cavities. Thus, in the kidney of *Tinca tinca*, Thélohan (1890, p. 200) has seen the tissue of the organ invaded while the tubes remained free (see also the above description of changes produced in the structure of the air bladder by the myxosporidium found in that organ).

#### 50. *Myxobolus* ? sp. incert. Pl. 22, fig. 4.

Psorosperms of *Cyprinus leuciscus*, Müller, 1841, Müller's Archiv., p. 486; *ib.*, Dujardin, 1845, Hist. Nat. des Helminthes, p. 614; *ib.*, Leuckart, 1852, Archiv. f. physiol. Heilkde, XI, p. 436, fig. 21c, d; *ib.*, Robin, 1853, Hist. Nat. des Végét. Parasites, p. 299.

*Synonymy.*—This is little more than a collection of references to spores found on "*Cyprinus leuciscus*." Robin's mention is, however, certainly the same as Müller's.

Cyst and myxosporidium unknown.

<sup>1</sup> Ztschr. f. wiss. Zool., 1881, XXXV, p. 633; Bronn's Thier-Reich, 1882, I, p. 598.

<sup>2</sup> Compt Rend. Acad. Sci. Paris, 1889, CIX, pp. 920-1.



*Spore*.—Resembling *Chloromyxum dujardini*; 11  $\mu$  (0.0051''') long and 7  $\mu$  (0.0034''') broad.

*Habitat*.—On *Leuciscus* (*Squalius*) *grislagine* L. (= *Cyprinus leuciscus*). Tumors less common than on *Leuciscus rutilus*.

It seems strange that Müller should approximate this form to the "sharp corpuscles of *C. rutilus*,"<sup>1</sup> as Leuckart's figure resembles much more closely the elliptic form figured by Müller (Müller's figs. *f*, *g*; pl. 28, figs. 5*f*, *g*).

51. *Myxobolus* sp. incert. Pl. 22, figs. 5, 6; pls. 23-25.

Barbel "psorosperms," etc., of—	mülleri.*	Date.	Authority; reference.
×	-----	1885	Méguin, Bull. Soc. Zool. France, X, pp. 351-2 (fig.); Compt. Rend. hebdom. Soc. Biol. Paris, II, pp. 446-7.
×	-----	1886	Railliet, Bull. et Mém. Soc. Centrale Méd. Veter. Paris, IV, pp. 134-7.
-----	Myxobolus { (pars).	1889	Ludwig, Jahresber. rhein. Fisch.-Ver. Bonn, 1888, pp. 27-36.
×	-----	1890	Railliet, Bull. Soc. Central. d'Aquicult. Paris, II, pp. 117-20.
×	-----	1890	Pfeiffer, Virchow's Archiv. f. pathol. Anat. u. Physiol., CXVII, pp. 552, 557-8, pl. 12, figs. A2, C1-8.
×	-----	1890	Die Protozoen als Krankheitserreger, 1 ed., pp. 28-9, 55, 67, fig. 10, plate, figs. IV, V.
×	-----	1891	Pfeiffer, Die Protozoen als Krankheitserreger, 2 ed., pp. 100, 105-10, 130, figs. 43 <i>b</i> , 45, 57.
×	-----	1892	Thélohan, Bull. Soc. philomat. Paris, IV, pp. 168, 178.
×	-----	1892	Henneguy and Thélohan, Annal. de Microgr., IV, p. 619.
×	-----	1893	Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, V, pp. 267-70.
×	-----	1893	Pfeiffer, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 118-130, plate, figs. 13-15, 16 (pars).
×	-----	1893	Sticker, Archiv. f. Animal. Nahrungsmittelkde Wien, VIII, p. 124.
Myxobolus.	-----	1893	Railliet, Traité de Zool. Méd. et Agric., pp. 158-159.

\* Non Bütschli.

† Ludwig's figures seem as though they might be generalized composites based upon several of Bütschli's. They may thus perhaps be not independent figures of the spore habitant in the skin of *B. barbus*, but have been considered to represent that form in view of its supposed identity with *M. mülleri*.

*Synonymy*.—Both Méguin and Ludwig, the former with doubt, the latter apparently without hesitation, regard this form as identical with *M. mülleri*. While admitting their superior advantages (of direct observation of material) I still feel considerable doubt as to the identity of these 2 forms, and have therefore provisionally classed them separately, as, while I do not consider that there is sufficient ground for a positive assertion of the distinctness of the two forms, there is certainly sufficient to justify a hesitation as to their fusion.

Méguin says the present species is probably the same as that described by Robin and Balbiani as infesting the tench and carp. Now as to this: (1) I am not aware that Robin ever observed such a form, and (2) the spore habitant on the tench (*M. ellipsoides*) is, as shown by Thélohan,<sup>2</sup> unquestionably distinct from that habitant on the carp (*M. oviformis*).

<sup>1</sup> "Bei *C. leuciscus* glichen sie ganz den spitzen Körperchen des *C. rutilus*."

<sup>2</sup> Annal. de Microgr., 1890, II, p. 210.

Further, Mégnin's figures would not by themselves induce me to fuse the two forms.

Besides, after considerable study of Ludwig's description, I am unable to decide how much of it represents his own observations and how much is copy of Bütschli's description of *M. mülleri*. It seems to be part original and part copy, but how much of each it is impossible to determine. It would seem as though Ludwig first determined in his own mind the specific identity of the present form (*M. sp. 51*) with *M. mülleri* and then applied to the former (*M. sp. 51*) Bütschli's description of *M. mülleri*, at the same time incorporating therewith certain observations, *e. g.*, the dimensions of the spore which must be his own (made upon *M. sp. 51*) inasmuch as they are not, to my knowledge, to be found in any previous description of *M. mülleri*. My reason for this view of the subject is Ludwig's statement that—

I can only confirm Bütschli's results upon the finer structure of *Myxobolus*.

Further, his figures bear some indication of being semidiagrammatic generalized composites of several of Bütschli's figures of *M. mülleri*. And still further his description (except the few additions) is Bütschli's. This course has rendered it impossible for me to distinguish how much of the composite description represents Ludwig's actual observations on *M. sp. 51* and how much of it merely pertains to *M. mülleri* generally, and is regarded as applying to *M. sp. 51*, by virtue of its supposed identity with *M. mülleri*. Under these circumstances I have credited to *M. sp. 51* only the minimum (*viz.*, the residual after subtracting from the composite, Bütschli's description of *M. mülleri*); as, though this residual may be incomplete for *M. sp. 51*, it is all that can be positively asserted to belong to that species.

Pfeiffer's figures (pl. 25, figs. 5, 6) approximate the present form much more closely to *M. ellipsoides* than to *M. mülleri*.

Finally, Thélohan says that the present species—

Presents a great resemblance to *M. mülleri*; perhaps it should, however, be considered as specifically distinct.

*Cyst.*—Membrane thin, probably formed by host. Contents clear living protoplasm, in which are imbedded very fine dark granules, very small nuclei corresponding to those of true cells, and spores (Ludwig).

Composed of an irregularly concentric-fibered layer inclosing a second double-contoured layer, which latter surrounds the cyst cavity filled with spores. The large white, stout-walled, walnut-sized, or smaller muscle cysts are situated near the skin or pleura; 30, 40, or more myxosporidia occur near together, surrounded by a loose web formed by the host. Each myxosporidium is to be regarded as an individual, and the multicamerate tubes result from the common encapsulating by the host of many such individuals of nearly equal age, which individuals subsequently, he thinks (from sarcosporidian analogy, etc.) fuse, the process recalling the so-called conjugation of the large free-living intestinal Gregarines (Pfeiffer).

*Myxosporidium*.<sup>1</sup>—Pfeiffer has seen the exit of the sporoplasm. He did not have the opportunity to cultivate the spores *via* the overhanging drop, but says such cultivation would be easy and would show the stage at which infection occurs. He did not actually see the myxosporidium penetrate the muscle cell, but he has found within that cell all growth-stages of the myxosporidium. The elongate myxosporidia often show, in their center, pansporoblasts containing well-developed spores, while at the ends these structures are smaller and contain only 1, 2, 4, or more nuclei. This proves that, as in the *Sarcosporidia* (also with the tubes of *Sygnathus* and, *fide* Thélohan, with those of *Cottus scorpio*), growth takes place at the ends of the tubes. Have these younger developmental stages originated from germs from the interior of the large tube, do they proceed from residual germs of the first multiple infection, or do they develop from newly immigrated germs? A positive answer can not yet be given, but in the barbel Pfeiffer regards the second mode (*viz*, a supplementary outgrowth from the germs which penetrated *en masse* in the first infection) as the more probable. In the myxosporidium tubes germs migrate from the center to the circumference, where they find better food conditions and through progressive division become new pansporoblasts (*Sporenkugeln*). The center of the cyst is also empty in the cysts of the sheep, those of the tench's air bladder, and that of the kangaroo's intestine. When the myxosporidia have attained a certain size, they are found free in the interstices of the muscular fiber. When crowded, they fuse to an irregular mass; only at the edges are some unfused myxosporidia to be seen. Hæmatoidin crystals are found in the myxosporidium.

*Spore formation*.—This appears in the smallest circular cysts with 16 to 20 germs; also in uniloculate elongate cysts thickly filled with 100 to 200 germs. In places large granule cells are imbedded in the muscular fiber. At another (? later) stage the dancing granules have vanished and the contents of the cells have separated with 10 to 20 or more pale globules one-third the size of the ripe germs. Also some fibrillæ show in their interior well-developed spores, with capsules and nuclei, single or in rank and file (? accident; ? pressure on cover-glass). The possibility of these must be admitted, yet the contents of the capsules appeared to have been voided.

*Spore*.—Lenticular or oval; length 12  $\mu$ , breadth 10  $\mu$ , thickness 6  $\mu$  (Ludwig); bivalve, shell cavity containing sporoplasm and 2 capsules, the latter extruding filaments under the influence of potassium hydrate (Mégnin); by glycerin (Pfeiffer).

Have the *Myxosporidia* resting spores? Mega-, and micro-spores (differing only in size) occur; also defective spores with 1 capsule, with caudiform appendages, or with a subrotund form (Pfeiffer).

*Habitat*.—Encysted and free in muscles, mostly of belly and sides of body (never elsewhere, the liver, spleen, ovary, eggs, and gills being

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<sup>1</sup> Description, Pfeiffer's (*loc. cit.*, 2 ed., 1891, p. 106).

free) of *Barbus barbus* L. (barbel) from the Rhine, Mosel, and Saar, the barbels of the Elbe and Weser territory being free from them (Pfeiffer). Also once in heart cavity (Ludwig). In barbels from the Marne, probably also from the Aisne and Seine (Railliet). Balbiani failed to find "adult psorosperms" in the viscera in Mégnin's material (Mégnin).

Liver, kidney, spleen, connective tissue of various organs; found in ovary by Balbiani.<sup>1</sup> In one case the myxosporidia and spores were lodged in a sort of cavity in the connective tissue of the intestinal wall 10 cm. from the anus. They produced a very conspicuous thickening, almost completely obliterating the lumen.

*Pathology.—Tumors:*<sup>2</sup> A badly infected barbel showed about 40 tumors; fully 10 per cent of all the muscular fibers were filled with spores. This condition must have resulted from auto-infection. The tumors may soften to an irregular stinking abscess containing spores, wandering cells, and the large bacilli (Pfeiffer; see below under *Ulcers*).

Tumors, usually 10 to 15, ranging in size from a nut to a hen's egg, with a very resistant wall 1 to 1.5 mm. thick; hemispherical or slightly elongate; sometimes uniting into patches 17 to 20 mm. long by 7 or 8 mm. broad in fishes of 2.5 kilos (about 5 pounds) weight. Scales over tumor raised, easily detachable, finally falling off. Not all tumors open, some fishes dying before the ulcer stage.

Some fishes die without external tumors, these being found located in the viscera (Meuse; Railliet). Usually of walnut size; sometimes, however, 50 mm. long and 20 mm. thick, single or multiple, usually on belly or sides; filled with a yellow or caseous purulent mass (Mosel, Saar; *vide* Ludwig).

<sup>1</sup>*Fide* Thélohan (Annal. de Microgr., 1890, II, p. 200; Compt. Rend. hebdom. Soc. Biol. Paris, 1893, v, p. 268) who refers to Balbiani's *Léçons sur les Sporozoaires*. The only page of the last work to which the reference could apply is p. 147, and as M. Thélohan says (letter to author, 1893), Balbiani is there not at all explicit.

<sup>2</sup>The following notes of four cases are from Ludwig. The fish were taken alive from the Mosel above Trier, died en route, and were examined the next day:

1. ♂ 30 cm. long; on left side just above ventral fin a tumor 50 mm. long, 40 mm. broad, and 30 mm. thick, extending above lateral line; skin and omentum in neighborhood of tumor normal.

2. ♀ 47 cm. long; two tumors: (a) on right side above ventral fin, under trunk muscles (which latter were, around the tumor, reddened), 45 mm. long, 35 mm. broad, and 15 mm. thick; covered by normal skin. Tumor so extended into body cavity as to have driven the omentum hernia-like before it. (b) On left side in front of pelvic bone, length 50 mm., breadth 15 mm.; already opened; orifice 10 mm. in diameter with an irregular strongly reddened border, surrounded by reddened skin. Cavity of ulcer filled with bloody mucus, which, apart from the admixture of blood, agreed with the tumor contents.

3. ♀ 44 cm. long; on left side at level of lateral line, between ventral and anal fins, a tumor 25 mm. long, 12 mm. broad, and 12 mm. thick; heart cavity filled with same substance as tumor contents.

4. ♂ 30 cm. long; in front of left ventral fin a tumor 35 mm. long, 25 mm. broad, and 25 mm. thick, projecting but little externally, but greatly into abdominal cavity.

*Opening of the tumors:* The active agents in the puriform transformation and opening of the tumor are the bacilli first observed by Pfeiffer in the ulcer contents. These are only found in the myxosporidian-infected muscles, never in other organs. The presence of these microbes either prevents connective tissue proliferation entirely, or prevents it from becoming complete, the tissue undergoing gangrene (a digestion-liquefaction, so to speak), which soon results in the destruction of the overlying tissues.

Subsequently the bacilli were studied by Thélohan (see synonymy, 1893) who observed two kinds of them:

1. Bacilli: Large, motile, as long as the spores, showing with hæmatoxylin 4 or 5 red granules, and a short flagellum; frequently several cohere by their surfaces; also long separated threads occur (Pfeiffer, 1891, p. 105).

Length  $6\ \mu$ ; sometimes isolated, sometimes in linear colonies, no motion seen; rapidly liquefying gelatin upon which it gives large, slightly yellowish-white colonies; in rabbits provoking a small, very limited abscess; staining easily with methylen blue, gentian violet, fuchsin, etc. (Thélohan, 1893).

2. Cocci: More rarely, sometimes with last, sometimes alone, another species consisting of Cocci isolated or united under the form of Streptococci or Diplococci occurs.

*Ulcers:* The tumors subsequently soften and burst, forming deep crateriform bloody-bordered ulcers filled with a yellowish purulent mass consisting of spores and of cell detritus. Among the latter large bacilli crawl.

*Cell infection:* The primary seat of infection is the interior of the muscle cell. Myxosporidia are found within well-preserved (distinctly transverse-striate) or markedly atrophied muscular fibrillæ; also between healthy fibrillæ. Atrophied muscle-cells are seen containing long rows of well-developed spores, which, on account of the absence of filaments within the capsules, Pfeiffer inclines to believe have reached their present position by a general immigration. In places the fibrillæ are beaded, such muscle bead-strings being ordinarily heaped near together in the neighborhood of the hard cysts. Around the cysts the muscular tissue is infiltrated with blood, the infiltration, where superficial, being visible through the skin. Near the ulcers the muscular substance is broken up, loosened, fatty-degenerated, and contains blood-colored tubes with numerous myxosporidia not yet encapsuled and also well-developed spores.

Thélohan<sup>1</sup> says:

In the ovary they are very frequently encountered. M. Balbiani has studied them in the ovary of the barbel and he has seen that the psorospermic matter does not confine itself to traveling *via* the connective tissue, but often invades the young ova.

*Pathological anatomy.*<sup>2</sup>—The presence of the parasite in the primitive muscle fiber seems to lead rapidly to degeneration. On examining

<sup>1</sup> Annal. de Microgr., 1890, II, p. 200.

<sup>2</sup> Description Thélohan's (Compt. Rend. hebdom. Soc. Biol. Paris, 1893, v, pp. 267-270).

fragments in the fresh state, fibers are seen, which, in places, have preserved their normal aspect and their striation, and at other points more or less considerable spaces, where the muscular substance is filled with a vitreous refringent mass, around and in the intervals of which lie fatty droplets, yellowish granules, and spores. The degeneration invades gradually the muscular substance of the primitive fibers, and one finds it in parts of these elements, where the parasite appears not to have penetrated. On the contrary, the neighboring, noninfested, primitive fibers seem exempt from that alteration, and one frequently observes a degenerated fiber surrounded by healthy ones.

The fiber thus degenerated and broken up, is soon invaded by phagocytal cells coming, some from the sarcolemma, others from the connective tissue. This latter, at the diseased points, is the seat of a very marked irritative proliferation.

It is necessary to distinguish, in the degenerated fiber, the parts where spores are found in great number, and those where these elements are few or absent, the degenerative process in the latter case having originated from the presence of the parasite at a different point.

In this latter case the cells which have penetrated into the degenerated tissue multiply rapidly; in proportion as their number augments, one sees the muscular débris diminish; very soon they have completely disappeared, the place of the fiber being finally occupied by connective tissue. While these phenomena occur, the irritation is propagated, the connective-tissue proliferation extends itself, and a sclerosis of the neighboring muscle region, with atrophy of the primitive fibers, is produced.

At the points where the degenerated fiber incloses a great number of spores, the formation of connective tissue is at first limited to a thickening of the perimysium. There are thus formed connective-tissue bridges, separating the spaces occupied by the spores, and which correspond to disappeared primitive fibers. These facts are seen especially clearly on transverse sections. Little by little these bridges increase in thickness, at the same time their tissue becomes more dense; they thus form around each space a fibrous shell, which tends to contract more and more. There seems to be here a true encystment of the parasite, such as is produced around foreign bodies introduced into the tissues.

*Symptoms.*—Barbels attacked are less lively than usual and have much difficulty in ascending streams; surface of body, dull, grayish yellow, oily, slippery (Meuse; Railliet).

Less lively than usual, easily caught in the hand, breasting the current with difficulty, avoiding rapid water (their usual haunt), taken in great numbers in bow-nets. Some affirm, others deny, that the sick fish will not bite at the hook. Diseased fish are of all sizes. Those seriously affected are of a weight much below that indicated by their external appearance, the body being in fact more or less dilated. On

this account the fishermen often estimate the weight at nearly double the actual (Railliet.)

According to Vet. Surg. Hanzo, the affected fishes float on the surface as though poisoned with *Cocculus indicus*.

*Epidemics.*—In the Meuse it has manifested itself with the characters of a veritable epidemic during three consecutive years, from 1883 to 1885, inclusive. It became progressively more aggravated, reaching its maximum of intensity towards the middle of 1885. On certain days of that year M. Ladague had interred nearly 100 kilograms of barbels; the Meuse was covered with dead fish. The disease subsided little by little, and actually appeared to become extinct, but it could almost be said that the combat closed for want of combatants.

In the district of Ardennes it was remarked only in the Meuse itself; all the affluents have always been spared. The maximum intensity, according to Railliet, was reached about the middle of 1884. On certain days, at Mézières alone, as many as 100 kilos (about 200 pounds) were interred. Some years later the disease had disappeared from that region, but raged down stream at Monthermé and Givet.

In the neighborhood of Nancy the barbels die in great numbers (Mégnin).

In the Aisne Railliet was informed of ravages of the disease occurring near Rethel. The disease, he thinks, extended to the Aisne and the Marne from the Moselle *via* the canals.

In the Marne a considerable number of barbels floated dead or unable to escape, down the lower Marne. The disease appears to have begun (at least in the neighborhood of Charenton) about June 15; thence it progressively increased, attaining its maximum at the time of emptying of the St. Maurice Canal. It persisted till the end of July, at which date Railliet's information ceased.

In the Seine it did not extend above the Port à l'Anglais dam. The Grenelle fishermen, Railliet was informed, had seen a great number of sick barbels. The Seine thus appears invaded, without doubt consecutively, from the Marne.

In the Rhine and its tributaries, the Saar and Mosel, according to Ludwig, it seems to have appeared at least several decades ago without, however, ever having attained the magnitude that it has reached in late years in the Mosel. The disease has there been observed since the end of 1870 and has so increased that, especially in the warm summer months, the dying and dead fish from the upper Mosel and Saar pass Trier by the hundreds, and at Zell (on the Mosel) it is reported that they spread a carrion-like odor. According to Pfeiffer, in the Saar and Mosel during the summer of 1890 no very extensive mortality occurred.

*Contributory causes.*—As regards age as a predisposing factor, Railliet observes that in the Meuse the young barbels are attacked as well as the old, the weights of dead fish varying from 22 grams to 6 or 7 kilograms.

In the 3 German streams Treplin<sup>1</sup> believes 3 series of cases to be distinguishable: (1) Mostly small fish (up to 100 grams), still well nourished, with only individual, or without recognizable, indurated patches, and which present in the abdominal region, at most, 1 hard tumor. (2) Somewhat larger fish (up to 200 grams), which almost always show in several places on their sides hard, somewhat swollen, patches; also tumors similar to those on the smaller fishes, mostly on the abdominal region. These fishes already begin to emaciate. (3) Fishes of and above the preceding weights, showing on the sides, belly, or back large ulcers, mostly lying immediately under the skin. A part of the same is already broken up; borders foul and red; interior containing a yellow pus. The fishes have emaciated greatly, and die.

Season, Railliet thinks, appears to have no influence, fish being seen dead in midwinter as well as in June, July, and August.

Pollution of streams Railliet considers a minor factor, saying:

The diversion into the Meuse of manufactory refuse is often blamed for the existence of this condition of affairs, but the investigations of M. Ladague tend to incriminate rather the erection of dams at certain points on the river, these structures diminishing the rapidity of current, in the midst of which the barbel ordinarily lives.

Treplin<sup>1</sup> believed that the young barbels receive the germ from refuse deposits of industrial establishments (breweries, malt houses, tanneries, distilleries, etc.) on the headwater of the Saar and Mosel; and, further, that these germs enter by the alimentary canal, passing thence into the rest of the body, and first make their exit therefrom (*via* the ulcers) in the second or third year. Herr Hanzo,<sup>2</sup> on the contrary, considers the cloth and paper mills as chiefly responsible, as these establishments handle old rags which are, he says, saturated with infective material.

Of the views of Treplin and Hanzo, Ludwig considers that of Treplin to have the greater degree of probability. Both, however, he remarks, consist only of opinions and probabilities, and further leave out of sight other sources of contamination. While no sufficient evidence exists for holding pollution of water by different industrial establishments responsible for barbel myxosporidiosis, an indirect connection between such water pollution and the disease is by no means to be entirely rejected. It is very easily possible that such pollution may favor myxosporidian increase and development, and especially that it may, by injuriously affecting the general life conditions, diminish the normal resistive power of the fish, thus rendering infection more easy. This view explains the fact (*fide* the fishermen) that the barbels at Bonn recover, while they die in the Saar and Mosel, in which latter streams pollution must, on account of the smaller volume of water, affect the fish more injuriously.

M. Braun<sup>3</sup> places less stress upon fouling of the water, as once

<sup>1</sup> In Ludwig, Jahresber. rhein. Fisch.-Vereins, Bonn, 1888, p. 34.

<sup>2</sup> In Ludwig, *loc. cit.*, pp. 34, 35.

<sup>3</sup> Review of Ludwig in Centralbl. f. Bakt. u. Parasitenkde, 1889, v, p. 420.



healthy whitefish sickened from introduction into water in which a whitefish affected with myxosporidiosis had died, and as the same disease is not rare upon *Coregonus* from lakes Peipus and Ladoga.

*Exciting causes.*—This may be safely assumed to be the presence and development of the myxosporidia. Pfeiffer,<sup>1</sup> from numerous examinations, states that these latter are always present in barbels from the Rhine, Mosel, and Saar, becoming pathogenic only at irregular intervals, probably when other causes so diminish fish vitality that the reactive encapsuling of the parasite is no longer possible. The latter then obtains the supremacy, and through the accompanying bacteria rapid death of the fish may result.

Mégnin's opinion is as follows:

*Mode of infection.*—One now understands how the fish become infected; the psorosperms which escape from the ulcers are ingested with the water during deglutition or respiration; under the form of an amœboid they enter the circulatory current, then arrive in the subcutaneous cellular tissue, which is their seat of election, where they undergo their last transformations.

Upon this subject Ludwig remarks that—

The greater frequency of occurrence upon the branchiæ suggests that infection occurs less through the alimentary canal than through the respiratory tract. The lymph paths of the connective tissue appear to represent the principal channels by which the parasite spreads through the body, but nothing certain is known.<sup>2</sup>

The infection of previously healthy fishes is brought about, Pfeiffer remarks, through the extensive fouling of the water by the numerous fish corpses, and the durable construction of the spores. Infection may then take place *via* the stomach, gills, or wounds. The last are of frequent occurrence in the spring at the time of breaking up of the ice.

*Remedies proposed.*—"How, now, to arrest the epidemic? It is difficult. I see no other method than to collect all the dead or sick fishes and destroy them by fire" (Mégnin).

Ludwig thinks that our ignorance of the complete life-history of the parasite, and especially of the way in which it secures a lodgment in the fish, precludes rational radical measures and permits us only to adopt certain prophylactic makeshifts. With reference to myxosporidiosis, as also for a number of other reasons, the waters, especially the Saar and Mosel, should be maintained in the highest state of purity, and to that end all pollution of the rivers mentioned, by communities or industrial establishments, should be interdicted. That most dangerous contamination of the water, by the *Myxosporidia* from the ulcers, cannot, of course, be stopped entirely, but it is evident that it will be less if all fishermen are impressed with the importance of destroying<sup>3</sup> all diseased and dead fish, instead of throwing them back into the water. Such destruction must be so effected as to prevent the reëntry of the germs into the water.

<sup>1</sup>Die Protozoen als Krankheitserreger, 1890, 1 ed., p. 67; 2 ed., 1891, p. 110.

<sup>2</sup>No actual observations are cited in support of this lymph-path theory.

<sup>3</sup>Pfeiffer (*loc. cit.*, 1 ed., 1890, p. 37) quotes Ludwig as recommending that they be buried.

Railliet (loc. cit., 1890) further says that every one up to the present appears to be in accord as to the means of combating the disease. It is, above all, expedient to collect the diseased fish and to bury them at a certain depth and at a great distance from the water course. This is what was done on the Meuse and one has just seen that this course succeeded sufficiently well. Thus at the end of some years the disease appears to have left no traces. Thus Railliet saw taken, even at Mézières, 3 barbels, the smallest of which weighed 1.5 kilos or 3 pounds.

Pfeiffer<sup>1</sup> says that prophylaxis must obviously be directed to the careful removal of all fishes dead of the disease. They should be burned or buried with caustic alkali. By this means, perhaps, the extermination of the barbel may yet be prevented.

The only attempts at cure are cited by Railliet, who says that M. Ladague succeeded by opening the tumors in greatly prolonging the life of the fish, and sometimes in curing it. If, on the contrary, the disease is allowed to take its course the tumors increase rapidly and the fish soon dies.

**52. *Myxobolus* ? sp. incert.** Pl. 26, fig. 1.

Psorosperms of *Cyprinus erythrophthalmus*, Remak, 1852, Müller's Archiv., pp. 144, 149, pl. 5, fig. 9B.

*Spore*.—Tailed and untailed were seen.

*Habitat*.—From pigment follicles on wall of splenic artery of *Leuciscus* (*Scardinius*) *erythrophthalmus* L.

*Remarks*.—As the relation between this form and *Chloromyxum dujardini* is at present doubtful, the present form is provisionally left separate.

**53. *Myxobolus* sp. incert.** Pl. 26, fig. 2.

Globules of *Cyprinus phoxinus* Rayer, 1843, Rayer's Archiv. de Méd. comp., I, pp. 58-9, pl. 9, fig. 13.

*Cysts*.—In the single specimen observed, 2 in number, yellowish white, the size of a pin's head; contents, a mass of ovoid spores. Ether rendered the cyst contents more transparent, ammonia more cloudy.

*Myxosporidium* and spore unknown.

*Habitat*.—Encysted on left side of head of *Phoxinus phoxinus* L., from the Seule River. Disease apparently rare.

**54. *Myxobolus oblongus* Gurley, 1893.** Pl. 26, figs. 3-6.

(Psorosperms of *Catostomus tuberculatus* (Le Sueur), Müller, 1841, Müller's Archiv., pp. 487-90, pl. 16, figs. 7-9; *ib.*, Müller, 1843, Rayer's Archiv. de Méd. comp., I, p. 229, pl. 9, figs. 7-9; *ib.*, Robin, 1853, Hist. Nat. d. Végét. Parasites, p. 301, pl. 14, figs. 9, 10.)

*Myxobolus oblongus*, Bull. U. S. Fish Com. for 1891, XI, p. 414; *ib.* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Myxosporidium* unknown.

*Cyst*.—Round or elliptic, not over 1 mm. in diameter; membrane

<sup>1</sup>Die Protozoen als Krankheitserreger, 2 ed., 1891, p. 110.

resistant; contents whitish, consisting of spores, with more or less granular detritus.

*Spore*.—Outline spatular, approaching roundish-oblong; untailed; length 14 to 17  $\mu$ , breadth 8.5  $\mu$ , thickness 5 to 6  $\mu$ .

Shell substance thin, almost perfectly transparent, insoluble in cold and moderately warm concentrated sulphuric acid, quickly destroyed when heated with the concentrated acid to near its boiling point; insoluble in concentrated solution of caustic potash, cold or hot. Valves separating in sulphuric acid (cold, concentrated), equally convex, the spore on transverse view appearing symmetrical on both (superior and inferior) sides of the wide ridge. (Greatest convexity of valves well forward (at about the junction of the anterior with the second fourth of the length;) ridge index nearly  $\frac{1}{3}$ ).

Capsules 2, pyriform, of equal size, containing a coiled filament visible (in iodine water) through the capsular walls; capsules drawn out anteriorly into the ducts, orifice visible. Methyl-green stains the capsular walls bright green; the filaments, sporoplasm, and shell not at all. Under this treatment there are differentiated in the uniformly bright green capsular walls several dark green granules. Sometimes only 2 are seen, and these are then often situated approximately in the long axis of the capsules. Other specimens are seen with 4 or 5, which are usually arranged without marked regularity, generally, however, being collected near the center. Their nature is problematical. Their presence, position, and numerical range appear to be constant.

*Sporoplasm*: The outline was not accurately traced, but the results, obtained by staining, suggest that upon the superior surface it may perhaps extend to the anterior end of the shell; upon the inferior surface it only reaches the posterior ends of the capsules. Upon this view of the relations, the capsules would indent the *inferior* surface of the sporoplasm. A similar condition appears to have been observed in other species (pl. 34, fig. 3d). It is obvious that between the greater (but partial) anterior projection of the sporoplasm upon the superior surface in *M. macrurus*, and its complete anterior extension upon one surface in the present species, various transitions might occur, and I believe that this greater anterior projection affords, even in the absence of valvular inequality, a criterion for the discrimination of the superior from the inferior surface, the greater projection being always superior and the capsules always more or less inferior.

*Nuclei*: Besides the deeply methyl-green staining bodies in the capsular walls, 3 series of bodies, which have a constant position and stain with both carmine and gentian violet, occur. Those forming the first series have every appearance of being, and I believe are, nuclei. The second and third series are much more dubious, for if all the granule-like particles which stain with gentian violet are to be regarded as nuclei, the number of the latter must be reckoned as 1 or 2 score. I have, therefore, merely described the appearances presented by the

specimens, and will direct attention to the possibility of sporoplasmic degeneration having taken place.<sup>1</sup>

Series 1: Consisting constantly of 2 deeply-staining globules (best shown by carmine), always found in the median tongue-like process of the sporoplasm, usually disposed submedianly, one behind the other, though not infrequently obliquely or even transversely directed; often seen closely approximated, sometimes flattened on their adjacent sides.

Series 2: Forming 2 curved lines whose direction and position coincide in a general way both with the concave anterior margins of the sporoplasm, and also with the adjacent postero-inner border of the capsule; best stained by carmine. Each line is resolved by high powers into several deeply-stained dots; its outer end approaches so closely the usual position of the pericorncular nucleus that I suspect that this latter structure may form the last dot. Further, with one pair of such lines distinctly in focus, a second pair (parallel and slightly anterior to the first) can sometimes be seen. That this pair exists on another focus-plane becomes evident by change of focus, when it comes into distinct view, the first pair at the same time receding into obscurity. Finally, at the anterior median cornu a distinct deeply-stained granule is also sometimes seen.

Series 3: These chromatophile bodies are best shown by gentian violet. This reagent differentiated, besides the lightly tinted shell, three kinds of substances which stain, respectively, not at all, medium, and very dark. There is never any difficulty in distinguishing these from one another; that is, there are no transitions between the tints. The medium-stained portion is the general protoplasm. Without pronouncing such to be their nature, I may say that the dark-, and non-staining portions behave toward gentian violet precisely as would nucleolar and nuclear substances, respectively. Moreover, the order of succession (from the center of the space outward) is always deepest-staining, nonstaining, medium-staining, the nonstaining portions forming circular, oval, or slightly irregular spaces, which are delimited by a sharp, clearly defined border from the surrounding medium-stained protoplasm on the one hand, and from the inclosed deeply stained granules on the other.

As regards their location, though they often seem to, and apparently sometimes do, honeycomb the protoplasmic portion of the spore, they nevertheless show a decided tendency toward peripheral aggregation. In most cases there can be distinguished in the posterior two-thirds of the spores 2 zones, a more deeply stained tongue-shaped median, and a markedly lighter band-like circumferential portion. The latter is, by preference, the seat of the third series of chromatophile bodies. The

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<sup>1</sup> The fishes had been kept for years in rather weak alcohol and their condition of preservation was by no means perfect. Further, the results of staining with gentian violet were by no means constant, only a single slide serving as a basis for the description given. The action of carmine was less variable.

anterior end of each series appears usually to be (is?) formed by one of the pericornual nuclei. Sometimes these latter are the only ones to be seen. Almost always they are the largest. Starting anteriorly with these two, an increase may be traced up to 6 (3 on each side<sup>1</sup>), the 3 pairs being often subsymmetrically arranged. In cases of deficiency it is the posterior ones that are absent. These facts would seem to suggest a possible origin of the series from the two large pericornual nuclei.

Besides the structures already described, others more or less similar may be seen, especially anteriorly and in the higher (presumably also in the lower) focus-planes. Some of these show the same combination (deeply stained granules in unstained areas) as those already mentioned, but often no surrounding unstained areas were visible.

Vacuole: I could not detect this structure, but do not wish, on the strength of the material available, to positively assert its absence.

*Habitat, etc.*—Encysted immediately beneath the skin, on the external (scaleless) surface of the head, never elsewhere except twice in skin of body immediately behind head of *Erimyzon sucetta oblongus* (= *Catostomus tuberculatus* Le Sueur, *vide* Jordan and Drayton<sup>2</sup>), chub sucker. Apparently a scaly surface constitutes an almost impassable barrier for this species.

Observed on fish collected as follows:

U. S. Nat. Mus. Cat. No. 20105. Tributaries Fox River, Mississippi. Collector, Prof. S. F. Baird. Tumors very numerous on 2 specimens. Fish adults.

U. S. Nat. Mus. Cat. No. 20523. Kinston, North Carolina. J. W. Milner, collector. A single tumor on 1 fish; the latter rather young.

This species was not found in the following:

U. S. Nat. Mus. Cat. No. 20254. Near Piermont (?Pierpont) New York. Collector Prof. S. F. Baird. Fish half-grown.

U. S. Nat. Mus. Cat. No. 25573. Columbia, South Carolina, March 21, 1880. Collector, Col. Marshall McDonald.

The striking contrasts between the very great number of cysts present on the fish from Mississippi and their extreme rarity upon those found at the other localities is interesting. Data are, however, wanting for the proper appreciation of relative potency of geographic location, temperature, season, and age of the fish.

*Remarks.*—This species is, I believe, identical with the one described by Müller.<sup>3</sup> Although he states the branchiæ to be the principal seat of this species, I have only found it imbedded under the skin covering the head. The cysts found on the branchiæ, besides being distinguished

<sup>1</sup> I have not seen more than 3 nucleiform bodies (deep-stained granules in the midst of a non-stained area) on a side, though the number of deep-stained granules may be greater, 2 being sometimes found in one unstained space.

<sup>2</sup> Bull. 12, U. S. Nat. Mus., pp. 100, 145; var. *oblongus*, *vide* Prof. B. W. Evermann.

<sup>3</sup> Müller's description in brief is:

Cysts conspicuous, elongate, 2 to 4 mm. long, imbedded principally under mucous membrane of branchial lamellæ, also in that of the branchial chamber and in skin of head of *Catostomus tuberculatus* from North American rivers. Cysts found in all of the 3 fish examined, being in one case numerous.

by their *much* smaller average size, contain a quite distinct species (*M. globosus*) which is much smaller, subcircular, and with a much larger capsular index.

55. *Myxobolus lintoni* Gurley, 1893. -Pl. 26, figs. 7, 8; pl. 27.

(Psoerosperms of *Cyprinodon variegatus*, Linton, 1891, Bull. U. S. Fish Com. for 1889, ix, pp. 99-102, pl. 35, figs. 1-16; *ib.* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiii, p. 97.)

*Myxobolus lintoni*, Bull. U. S. Fish Com. for 1891, xi, p. 414; *ib.* of *Cypsinodon* [error] *variegatus* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Cysts*.—Apparently no closed cysts. Fungoid masses of an irregular shape, varying in size from 4 by 2.5 mm., to 10 by 4 mm., projecting as much as 3 mm. above general surface of skin.

*Myxosporidium* unknown.

*Spore*.—Shape and size very uniform; biconvex-lenticular, outline broadly rounded-elliptic, length 13.9  $\mu$ , breadth 11  $\mu$ , thickness about 8  $\mu$ . Shell thick, showing under action of osmic and sulphuric acids a low longitudinal ridge, resisting the action of concentrated sulphuric acid and of potassium hydrate solution and a 10 days' maceration in sea water; staining brown with iodine and deeply when treated with methyl green and eosin; collapsing under action of glycerin. Capsules 2, situated and converging anteriorly, pyriform, transparent, refractile, not staining deeply with methyl green and eosin, showing, with osmic acid, a minute pore at anterior end; containing filaments which are extruded under the influence of sulphuric acid; filaments when extruded nearly straight, undulate, or more or less closely spiral, of the same thickness throughout, distal ends attenuate. Sporoplasm showing, on addition of acetic acid or after 8 days' immersion in sea water, a "nuclear vesicle"; in many specimens showing the "smaller supplemental refractile bodies" represented in pl. 27, fig. 2. Spore associated with calcareous particles of irregular shapes (fig. 14).

The above is Prof. Linton's description, condensed and rearranged. To it I am able to add, partly by way of correction, the following data:

*Spore*.—Shell composed of 2 valves, superior and inferior; easily and rapidly separating in sulphuric acid (cold, concentrated); ridge present. Capsules extruding the filaments (alcoholic specimens) in a loose spiral or straight, under the action of iodine water. Sporoplasm showing, with iodine, a rather large vacuole with clearly defined borders. Nuclei, at the most, 4, 2 of which are the pericorncal.

These 2 specimens were also from the Atlantic, at Woods Holl, Mass.; collected by Mr. V. N. Edwards, August 1, 1892.

*Habitat*.—Imbedded in the subcutaneous tissue of *Cyprinodon variegatus* (short minnow), taken in the Atlantic at Woods Holl, on August 20, 1889; also August 1, 1892.

*Effects*.—The skin of the host overlying these tumors is more or less cracked and broken, and the scales scattering.

**56. *Myxobolus* sp. incert.** Pl. 28, fig. 4.

Cyst and myxosporidium unknown.

*Spore*.—Broadly elliptic; length,  $14\ \mu$ ; breadth,  $10\ \mu$ ; thickness,  $5\ \mu$ ; shell bivalve; valves equally convex; ridge index about 0.25. Capsules 2, equal; capsular index not quite 0.50. Sporoplasm showing a clear, round space, without doubt the vacuole.

*Habitat*.—Body cavity of *Carassius carassius* L. (goldfish), from Germany.

*Remarks*.—For this species I am indebted to Dr. C. W. Stiles, who mounted the spores in Leipsic. The exact locality whence the host came is unknown. The specimen was mounted unstained in Farrant's solution. For this reason the vacuole could not be stained or the nuclei be determined.

**57. *Myxobolus* ? *obesus*** Gurley, 1893. Pl. 28, fig. 7.

(Psorosperm of the "Ablette," Balbiani, 1883, Journ. de Microgr., VII, p. 203, fig. 43; *ib.* Balbiani, 1884, Léçons sur les Sporozoaires, p. 133, fig. 39.)

*Myxobolus obesus*, Bull. U. S. Fish Com. for 1891, XI, p. 415; *ib.* of *Alburnus lucidus*<sup>1</sup> Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

No description.

*Habitat*.—On *Alburnus alburnus* L.

**58. *Myxobolus* *cycloides*** Gurley, 1893. Pl. 28, fig. 5.

(Psorosperms of *Cyprinus rutilus*, Müller, 1841, Müller's Archiv., pp. 481, 486, pl. 16, fig. 4d-g; *ib.*,<sup>2</sup> Creplin, 1842, Wiegmann's Archiv. f. Naturgesch., I, p. 63 (footnote); *ib.*, Müller, 1843, Rayer's Archiv. de Méd. comp., I, p. 226, pl. 9, fig. 4d-g; *ib.*, Rayer, 1843, *ibid.*, p. 269; *ib.*, (pars) Robin, 1853, Hist. Nat. Végét. Parasites, p. 299, pl. 14, fig. 6.)

*Myxobolus cycloides*, Bull. U. S. Fish Com. for 1891, XI, p. 415; *ib.*, Braun, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Cyst*.—Not described. Creplin states that the membrane is very delicate and that it is "dissolved" by water.

Myxosporidium unknown.

*Spore*.—Subcircular-ovate or broadly rounded-elliptic, resembling *M. circularis*; length,  $12\ \mu$  ( $0.0054''$ ).

*Habitat*.—Encysted, most frequently on inner surface of opercle and particularly on the pseudobranchiæ (*Nebenkiemen*) of *Leuciscus rutilus* from German rivers. Disease of very frequent occurrence, principally in May and June. Creplin's specimens were taken May 8, 1835, and January 31, 1839.

**59. *Myxobolus* sp. incert.**

Myxosporidian spore of *Gardon*, Thélohan, 1889, Compt. Rend. Acad. Sci. Paris, CIX, p. 921.

*Spore*.—Vacuole present; maximum number of nuclei, 3.

*Habitat*.—On the "*Gardon*." At present this form is entirely indeterminate, as M. Thélohan informs me (letter, 1893) that *Gardon* is applied indiscriminately to both *Leuciscus rutilus* and *L. erythrophthalmus*.

<sup>1</sup>The question between the two specific names is merely that of the advisability of the use of a specific name identical with the generic.

<sup>2</sup>Creplin compares his form to Müller's, fig. 4d.

**60. *Myxobolus sphaeralis* Gurley, 1893.**

(Psorosperms of *Coregonus fera*, Claparède, 1874, in Lunel's Hist. Nat. d. poissons du bassin du Léman, pp. 113-14. )

*Myxobolus sphaeralis*, Bull. U. S. Fish Com. for 1891, XI, p. 415; *Myxobolus sphaeralis* [error] Braun, 1894, Centralbl. f. Bakt. u. Parasitenkunde, xv, p. 87.

*Cyst.*—Diameter, 0.25 to 0.33 mm.

Myxosporidium unknown.

*Spore.*—Very different from those contained in the cysts of the muscles of the same fish, untailed, perfectly spherical,  $9\ \mu$  in diameter, containing a single spherical, very strongly refringent "nucleus" and some small granules. Some cysts contain spores with less refringent nuclei and with very numerous small granules. This difference is perhaps only one of age.

*Habitat.*—Cysts imbedded by thousands in the mucosa of the branchiæ of *Coregonus fera* Jur. Their abundance gives to the branchiæ a grayish color apparent at the first glance.

*Remarks.*—Claparède remarks that it might naturally be supposed that a generic bond exists between the small cysts of the branchiæ and the large cysts of the muscles, but observation was unable to justify this hypothesis.

**61. *Myxobolus* sp. incert. Pl. 28, fig. 6.**

Psorosperms of *Lucioperca sandra*, Müller, 1841, Müller's Archiv., pp. 480-6, pl. 16, figs. 3a-l; *ib.*, Müller, 1843, Rayer's Archiv. de Méd. comp., I, pp. 222-6, pl. 9, fig. 3a-l; *ib.*, Dujardin, 1845, Hist. Nat. d. Helminthes, p. 644; *ib.*, Robin, 1853, Hist. Nat. d. Végét. Parasites, p. 295, pl. 15, fig. 5.

*Cysts.*—Flat white vesicles or pustules, 1.09 to 2.18 mm. ( $\frac{1}{2}$  to  $1\frac{1}{4}$ ) in diameter, usually few and discrete; contents a small quantity of granular matter, mostly, however, consisting of the spores.

Myxosporidium unknown.

*Spore.*—Almost exactly round, untailed or very rarely (once in 200 to 300 times) tailed, the tailed forms occurring in the same cyst and resembling especially *M. schizurus*, from which species, however, they differ in having the tail no longer or only a little longer than the body; with double-contoured border, thickness equal to one-half the breadth; ridge present; capsules 2, of equal size, converging and appearing as though united by a knot at their anterior extremities (fig. 6a). Among multitudes of typical specimens, Müller says an occasional one is seen containing 3 bodies, the third being placed behind and between the other two. Spore frequently showing a dark punctule just behind the posterior end of each capsule which sometimes simulates an oblique line extending from the border to the capsules; at others, a slight projection of the shell.

*Development.*—Traced (naturally enough, but erroneously<sup>1</sup>) by Müller, as follows: (1) Spores occur in which the capsules are no longer at the

<sup>1</sup>It must be remembered that Müller was not aware of the existence of the myxosporidium. Recently Miugazzini has attempted to revive this view of the office of the capsules (see p. 87).



anterior end, but in the middle, and have their axes parallel (fig. 3*h*). (2) Numerous mother vesicles [pansporoblasts] are seen containing 2 spores standing on edge, in contact, with their longitudinal planes parallel; such spores show capsules in their interior in the usual place. (3) Rare cases occur (fig. 6*e*) where the mother vesicles contain 3 such spores; these correspond to the rare cases in which the contents of the spore consist of 3 parts. He concludes that the capsules are the germs of new spores.

*Habitat*.—Encysted in skin of the external or internal surface of the opercles, in the rays of the branchial membrane, on upper surface of head or on the fins of *Stizostedion lucioperca* (= *Lucioperca sandra*), pike perch, from German rivers and from the Don. Disease very frequent, mostly in May and June. Müller found it in from 20 to 25 per cent of the young fishes examined. They were taken during the first of the winter.

62. *Myxobolus globosus* Gurley, 1893. Pl. 28, figs. 1-3.

Bull. U. S. Fish. Com. for 1891, xi, p. 415; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Cysts*.—Varying from very minute to a maximum of 0.5 mm., elongate-elliptic or rod-shaped, apparently (judging from ease of rupture) with a very thin membrane; color, whitish; contents, spores.

*Myxosporidium* unknown.

*Spore*.—Globose, subcircular in outline, untailed; length, 7 or 8  $\mu$ ; breadth, 6 or 7  $\mu$ ; thickness, 5  $\mu$ . Shell substance thin, very transparent, composed of 2 valves (superior and inferior in position), which present a heavy ridge whose width nearly equals one-third of the thickness of the spore. Valves equally and very convex on their external surfaces, appearing symmetrical on either side of the ridge. Capsules, 2, of equal size, rather strongly diverging; capsular index somewhat more than 0.50. Nuclei 3 or 4, viz: the 2 pericornual and 1 or 2 others, the latter the usual and presumably the fully developed condition (see p. 92). Vacuole present. Owing to the great convexity of the sporoplasm surface and the great thickness of its substance, it is not so clearly outlined as usual.

*Habitat*.—Encysted on the branchial lamellæ of *Erimyzon sucetta oblongus* Lac. (= *Catostomus tuberculatus* Le Sueur<sup>1</sup>), chub sucker.

This species was found upon fishes from the first 3 localities; on those from the fourth none were detected.

The following is the record of fishes examined:

U. S. Nat. Mus. No.	Locality.	Date.	Collector.
20523	Kinston, N. C. ....	.....	J. W. Milner.
25573	Columbia, S. C. ....	Mar. 21, 1880	Marshall McDonald.
20105	Tributaries Fox River, Mississippi	.....	S. F. Baird.
20234	Near Piermont († Pierpont), N. Y. ....	.....	S. F. Baird.

<sup>1</sup> Fide Jordan & Drayton, Bull. 12, U. S. Nat. Mus., pp. 100, 145; var. *oblongus*, fide Prof. B. W. Evermann.

63. *Myxobolus transovalis* Gurley, 1893. Pl. 29, fig. 1.

Bull. U. S. Fish Com. for 1891, XI, p. 415; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Cyst.*—Existence not evident, the spore-mass appearing to be held together by a small soft gelatinous or mucoid mass which has no attachment to the subjacent connective tissue, as it invariably comes away with the scale. It forms a thin discoidal mass situated in the center of the concave under surface of the scale. When at its thickest it elevates the scale slightly, and this elevation is the principal guide to its detection. In addition its color when coagulated is a slightly deeper yellow than that of the surrounding tissues. It is exceedingly difficult, in fact nearly impossible, to detect its presence in the fresh state.

*Myxosporidium* unknown.

*Spore.*—Length, 6  $\mu$ ; breadth, 8  $\mu$ ; shell thin; substance almost perfectly transparent, insoluble in concentrated sulphuric acid, bivalve; the valves superior and inferior in position, equally ventricose, with a narrow ridge; valves separating easily when placed in cold concentrated sulphuric acid, also sometimes in strong glycerin, or when the mass is rolled under the cover slip.

*Capsules:* Two, of equal size, containing a coiled filament extruded under the influence of glycerin and of sulphuric acid; capsular index about 0.50.

*Sporoplasm:* The great convexity of the sporoplasm renders it difficult of determination whether the deeper iodine-stained portions represent merely greater thickness or a vacuole. Sometimes the latter view was suggested by the rather sharp outline of such deeper-stained areas. Hydrochloric acid alcohol carmine stains 2 (very rarely 1 only) comparatively large (1 to 1.5  $\mu$  in diameter) nuclei, which are always and plainly situated in the sporoplasm with a site by preference along or near one of its concave anterior borders; pericornual nuclei apparently absent.

*Habitat.*—Under scales on external surface (mostly on posterior half) of *Phoxinus (Clinostomus) funduloides* Girard, taken in 4-mile Run (tributary of Potomac River), near Carlins, Va., June 29, 1892; collector, the author. Among fishes collected from the same locality, August 29, 1892, no diseased specimens were found.

64. *Myxobolus* ? *merlucii* Perugia, 1891. Pl. 29, figs. 2-7.

*Myxosporidium merlucii* Perugia, 1891, Boll. Scientific., Pavia, XIII, pp. 22, 24, figs. 9-14; *Myxobolus merlucii* [error], Thélohan, 1892, Bull. Soc. philomat. Paris, IV, pp. 166, 178; *M. merlucii*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 415; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Myxosporidium.*—Occurring under various forms; no differentiation of ectoderm; no pansporoblast membrane. The spores are expelled at their maturity from the myxosporidium. Perugia adds:

I have also seen form 2 contiguous vacuoles which do not present the slightest trace of capsules, but only a few granulations.

*Spore*.—Always 2, oval, with 2 capsules situated “at the superior border in the transverse diameter.” Perugia did not see the extrusion of the filaments under the action of reagents. He adds that he has convinced himself of the accuracy of Thélohan’s opinion as to the vacuolic nature of Bütschli’s “nucleus” and also of that of Thélohan’s observations upon the nuclei of the spore.

*Habitat*.—Gall-bladder of *Merlucius merlucius* (= *esculentus*, = *vulgaris*), hake, collected August 13, 1890.

*Remarks* (see also p. 275).—This is a rather peculiar species, and the generic reference is provisional. As indicated elsewhere, gall-bladder species of *Myxobolus* are so rare that this habitat is a caution-mark as to the generic reference of imperfectly described forms. The present generic reference is made provisionally and very doubtfully upon Perugia’s assertion of the presence of an iodophile vacuole. Finally, attention may be directed to Perugia’s figure 9 (pl. 29, fig. 2), which differs entirely from the others.

#### 65. *Myxobolus* ? sp. incert.

Psorosperms of *Gobio fluviatilis* Lieberkühn, Müller’s Archiv., pp. 353–4; *ib.*, Lieberkühn, 1854, Bull. Acad. Roy. Belg., XXI, pt. 2, pp. 21–2; ? myxosporidian of kidney of *G. fluviatilis* Thélohan, 1890, Annal. de Microgr., II, p. 198; *ib.*, of *Gobius* [error] Pfeiffer, 1890, Die Protozoen als Krankheitserreger, 1 ed., p. 49; *ib.*, Pfeiffer, 1891, *ibid*, 2 ed., p. 134.

*Cyst*.—Nearly spherical, about 0.22 mm. in diameter; contents, “psorosperms,” empty shells of the same, “free nuclei” of the same, and amœboid bodies with amœboid movements.

*Myxosporidium*.—The above and below mentioned amœboid bodies in all probability represent the earliest stages.

*Spore*.—Untailed. Lieberkühn repeatedly saw spores contract to an hour-glass shape and extrude an amœboid body, which formed blunt processes, and moved slowly over the field, the movements continuing for a long time; amœboid bodies diaphanous, destitute of granules and of apparent structure, usually invisible within the spore, but sometimes plainly seen; size, that of a colorless blood corpuscle.

*Habitat*.—In the kidney and encysted in body cavity between the kidney and the air-bladder of *Gobio gobio* L.

*Remarks*.—The habitat and the “encysted” condition of this form imply *Myxobolus* affinities.

#### 66. *Myxobolus* ? sp. incert.

Psorosperms of *Perca fluviatilis*, Müller, 1841, pp. 481, 490; *ib.* Robin, 1853, Hist. Nat. des Végét. Parasites, p. 296; *ib.* Lieberkühn, 1854, Müller’s Archiv., p. 365; *ib.* Bessels, 1867, Tagebl. d. 41 Versamml. d. deutsch. Naturf. u. Aerzte, pp. 71–72.

Cyst mentioned but not described by Lieberkühn; myxosporidium unknown.

*Spore*.—Untailed. Bessels observed the extrusion of the filaments as a result of 8 hours’ immersion in glycerin.

*Habitat*.—In May and June encysted in the skin of *Perca fluviatilis*

(yellow perch) in German rivers and in the Irtisch (Müller). Scales (Lieberkühn; Bessels). Disease not common.

*Remarks.*—Bessels's form seems probably referable here, as he speaks of having observed the longitudinal splitting into 2 symmetrical halves of an ellipsoid form.

**67. *Myxobolus* sp. incert.** Pl. 29, fig. 8.

Psorosperms of *Leuciscus rutilus*, v. d. Borne, 1886, Handb. d. Fischzucht u. Fischerei, p. 211, fig. 215.

No description.

*Habitat.*—On *Leuciscus rutilus* L.

**68. *Myxobolus* ?? *zschokkei* Gurley, 1893.** Pl. 31, fig. 1.

(Psorosperms of *Coregonus fera*, Zschokke, 1884, Archiv. de Biol., v, pp. 234-5, pl. 10, fig. 16; *ib.*, Linton, 1891, Bull. U. S. Fish Com. for 1889, ix, p. 101.)

*Myxobolus* ?? *zschokkei*, Bull. U. S. Fish Com. for 1891, xi, p. 416.

*Cyst.*—Oval, white, size varying from that of a small pea to that of a large nut; multiple, sometimes as many as 30 on one fish, the largest usually situated in dorsal muscles; cyst membrane thick, very resistant, without apparent structure; contents a milky fluid, occasionally a caseous mass, coagulable by alcohol.

*Myxosporidium* unknown.

*Spore.*—I quote in substance Zschokke's description:

Body lenticular or oval, a little wider in front than behind; often bearing in front a blunt prolongation; posteriorly one distinguishes 2 "tails" (*queues*), 6 to 8 times longer than the body, attenuating posteriorly, curved and undulating; the number of 2 "tails" is constant; at the pole opposite to the "tails" are 2 oval, transparent anteriorly-converging vesicles; one sometimes sees, however, an extremely fine canal extending from the posterior end of each vesicle to the base of the corresponding "tail"; the vesicles then probably play here also the role of receptacles for the "tails." Round refractile globules are also seen at the bases of the vesicles; the remainder of the body is filled by a homogeneous plasmic mass, which frequently contracts to the center of the body cavity, forming a clearly distinct round or oval mass.

*Habitat.*—Encysted in the subcutaneous and superficial intermuscular tissue of *Coregonus fera*. Observed during April and May. Disease stated by fishermen to be of very frequent occurrence.

*Effects.*—The skin is irregularly swollen and the scales fall easily. As to myxosporidiosis of *Coregonus*, see also p. 233.

This form is a very puzzling one. As appears from the above description and from the figure (pl. 31, fig. 1), the 2 structures, called by Zschokke "tails" (*queues*), are seen at one end, and at the opposite end are 2 structures (the "vesicles" of the above description) approximating to the position of and presenting somewhat the appearance usual to the capsules, and Zschokke considers them to be the capsules. They converge, as do the capsules of most species, toward the end of the spore, at or near which they are situated, and they diverge in the opposite direction. From these facts one would be inclined to pronounce this end (*viz.*, the one at which these "vesicles" are placed and toward which they converge) the anterior, and the opposite one (the

one from which the "tails" proceed) as the posterior. Zschokke, however, states that he has often seen a fine canal running from the (on the above supposition) *posterior* end of each capsule to the base of the "tail," and expresses his belief that, in this species as in those observed by Balbiani, the function of the "vesicles" is to contain the "tails." Both he and, subsequently, Linton<sup>1</sup> perceived the anomaly which, upon his view, is presented by this species, but neither of them discusses it at length. It is almost as difficult to reverse the position of the spore and consider the "tails" as corresponding to the filaments which in other species are extruded from the capsules, as this view would necessitate the admissions that the capsules are placed at and converge toward the posterior end of the body, and that the filaments are extruded from their posterior ends, a state of things occurring in no other known species.<sup>2</sup> I may add that the filiform aspect of the so-called "tails" is quite different from that shown by the stout tails of other species, while it closely resembles that of the capsular filaments.

69. *Myxobolus* cf. *creplini*. Pl. 30.

Myxosporidian spore of *Esox lucius*, Weltner, 1892, Sitzungs-Ber. Ges. Naturf. Freunde Berlin, 1892, pp. 28-36, figs. 1-16.

The fish was a spawner, weight estimated at 1 kilo; it showed a mass of milk-white eggs whose contents consisted of myxosporidian spores, a granular mass, and a few yolk granules. The material was first examined by Hilgendorf, who recognized the myxosporidian spores.

Spore dimorphous, untailed and tailed forms occurring. Anterior end more or less bluntly rounded. Posterior end showing great differences, as a rule gradually drawn out without any boundary into the thin tail. More rarely the alternation is sudden and the tail is then delimited from the body. With some spores there is found at the place of transition of the body into the tail a wing-like expansion, which lies at the border of the spore. The untailed spores have the posterior end rounded, much blunter than the anterior; otherwise they are formed entirely like the tailed. The tailed spores are of a fusiform shape.

Relation of untailed to tailed: It might readily be believed that the tailed develop from the untailed by the appearance of a short stump, which would subsequently grow in length and breadth; thus the body-length of the 2 forms is about the same, the whole length of the tailed consequently exceeding that of the untailed only by the length of the tail. Also the maximum width is about the same for both spore-forms.

Shell consisting of 2 thick almost always unequally arched<sup>3</sup> valves which can gape apart anteriorly for more than half their length; by

<sup>1</sup> Bull. U. S. Fish Com. for 1889 (1891), p. 101.

<sup>2</sup> *M. diplurus* has (if Bütschli's figure be correct, pl. 36, fig. 4) the capsules *posteriorly* placed, but their convergence and divergence is not evident, and nothing is known about the capsular filaments.

<sup>3</sup> Weltner refers to his figs. 8 to 11, in which the inequality of valve-convexity might perhaps be the result of the oblique positions of the spores.

pressure on the cover-glass they can be separated almost completely. They remain, however, connected at the posterior end; ridge present.

On longitudinal ("end") view the valves are seen to unite with each other, either by direct fusion and without appreciable line of demarcation, or to be soldered by the thick interiorly projecting welt-like ridge (in optical section, circular).

Weltner believes that the tail structure (in this species) always consists of a superior and an inferior half, each half being a process of the corresponding valve. For, in the very few cases in which the valves diverged posteriorly (remaining connected anteriorly), he saw this quite plainly; with some shells the tail-halves were shorter; with others longer; also inequality of length is very frequent in the same spore, and one valve-process may be very long and the other very short. Other spores have only one valve sharply drawn out, the other showing no trace of a tail. Tail thinner than that of *M. psorospermicus* (Lieberkühn's figures in Bütschli).

The spores in which the tail is double may lie in 3 positions:<sup>1</sup> (1) Most frequently the tails are plainly visible only on a transverse (or at least an oblique) view. The tail-halves (which on vertical view cover each other) then diverge. (2) With other spores things are different; here the tail-halves appear side by side, on vertical view. (3) The third position is that in which the tails cross (in the manner of a crossbill's beak) both on vertical and transverse views.

Capsules: 2, fusiform, length 5.1 to 5.9  $\mu$ ; their posterior end bluntly rounded off and often obliquely truncated.<sup>2</sup> The separated capsules are rounded pyriform. Capsules mostly parallel-appressed, mutually flattened. In spores whose capsules lie separated from each other the granulated sporoplasm is seen between them. Longitudinal ("end") views show the capsules to be imbedded in the sporoplasm. Weltner only once certainly observed the sporoplasmic covering to extend as far forward as the apex of the capsules. The latter is always clear and glistening when containing the filament; dull when empty. The capsule of the present form differs from that of *M. psorospermicus* (Lieberkühn's figures in Bütschli) in shape; also here the capsular index is smaller. In *M. schizurus* the shape and position of the capsule is also different.

Filament: Not visible (under a power of 1,000 diameters) through the capsular wall; only a dark shadow being seen. Exit produced by glacial acetic acid; also (spores in alcohol), by pressure on the cover glass; the last method produced the extrusion of many filaments; extruded filaments often quite straight; length, 47.9  $\mu$ .

<sup>1</sup> It seems to me that all this is produced merely by a slight lateral shifting of the valves and by the flexibility of the tail. At any rate all these aspects are so produced in *M. cf. linearis* (see p. 254).

<sup>2</sup> A similar apparent marked truncation is an optical illusion in *M. macrurus*.

**Sporoplasm:** In the preparation this had run to a mass with plainly visible coarser and finer granules. Sporoplasm traceable only to the root of the tail, where its lateral borders converge sharply; in the untailed forms it is rounded off posteriorly.

No nucleus was discovered; bodies staining with hæmatoxylin, borax-carmin, bismarck brown, gentian violet and *Kernschwarz* were resolved by a Leitz  $\frac{1}{20}$  immersion into coarse granule-heaps, having little similarity to nuclei.

**Microscopic technique.**—Material received fresh; the pathologic material was placed in glycerin and water (equal parts) and fixed with some drops of saturated sublimate solution; 14 days later it was transferred to 50 per cent, and subsequently to 70 per cent alcohol. In alcohol the eggs remained soft. In this form the material was catalogued as *Protozoa* No. 1661 in the collection of the *Königliches Museum für Naturkunde*. Bismarck brown stains the capsules only; borax carmine, only the sporoplasm.

**Habitat.**—Ovary of *Lucius lucius* (pike) collected at the beginning of February, 1892.

**Remarks.**—Of Müller's forms, the present species resembles most, but is not identical with, *M. schizurus*. This species also bears a great similarity to Lieberkühn's figures (in Bütschli) of *M. psorospermicus*, but here too, specific differences exist. On the contrary, he believes the present form to be identical with *M. creplini*, as the shape and size of the two agree well; it is, however, to be noted that the thickness is seldom as great as that of the last-named species.

#### 70. *Myxobolus brevis* Thélohan, 1892.

(Cf. tailed psorosperms of kidney of *Gasterosteus aculeatus*, Lieberkühn, 1854, Müller's Archiv., 1854, p. 357 (see p. 185); myxosporidian spores of *G. aculeatus* and *G. pungitius* (pars) Thélohan, 1890, *Annal. de Microgr.*, II, pp. 198-200, 209, pl. 1, fig. 1; *ib.* (pars) Thélohan, *Compt. Rend. hebdom. Soc. Biol. Paris*, II, p. 604.)

*Henneguya brevis* Thélohan, 1892, *Bull. Soc. philomat. Paris*, IV, p. 177.

*Myxobolus brevis*, Gurley, 1893, *Bull. U. S. Fish Com. for 1891*, XI, p. 416.

*Henneguya brevis*, Braun, 1893, *Centralbl. f. Bakt. u. Parasitenkde*, XIV, p. 739.

*Myxobolus brevis*, Braun, 1894, *Centralbl. f. Bakt. u. Parasitenkde*, xv, p. 87.

Cyst and myxosporidium not mentioned.

**Spore.**—Small; length, 15  $\mu$ ; breadth, 5 to 6  $\mu$ ; anterior portion more swollen; tail very short, caudal index hardly 0.50.

**Habitat.**—Renal tubules and ovary of *Gasterosteus aculeatus* (stickleback); renal tubules and ovary of *Pygosteus pungitius* (9-spined stickleback); all *vide* Thélohan, letter, 1893.

**Effects.**—The following from Thélohan probably refers to this species:

At the moment of the expulsion it is not rare to see the normal spawning replaced by the expulsion of a small mass of gluey and viscous matter in which the microscopist easily recognizes psorosperms, aborted eggs, etc.

**71. *Myxobolus medius* Thélohan, 1892. Pl. 31, figs. 2-4.**

(Cf. tailed psorosperms of kidney of *Gasterosteus aculeatus* Lieberkühn, 1854, Müller's Archiv., 1854, p. 357 (see p. 185); myxosporidian spores of *G. aculeatus* and of *G. pungitius*, Thélohan, 1890, Annal. de Microgr., II, pp. 198-200, 209, 211, pl. 1, figs. 1, 18 (last *vide* Thélohan, letter); *ib.* Thélohan, 1890, Compt. Rend. hebdom. Soc. Biol. Paris, II, p. 604.)

*Henneguya media* Thélohan, 1892, Bull. Soc. philomat. Paris, IV, p. 176.

*Myxobolus medius* Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 416.

*Henneguya media* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739.

*Myxobolus medius* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

Cyst none; myxosporidium unknown.

*Spore formation*.—Pansporoblast apparently monosporogenetic (see pl. 31, fig. 4, reproduction of Thélohan's fig. 18).

*Spore*.—Fusiform; length, 20 to 22  $\mu$  (Thélohan, 1892); total length, 24 to 30  $\mu$  (*ibid.*, 1890); shell striate; tail present, resembling especially that of *M. psorospermicus*, curved close against the body during development, straightening only after rupture of the pansporoblast membrane; nuclei unknown; vacuole present.

*Habitat*.—Renal tubules and ovary of *Gasterosteus aculeatus* L. (stickleback); renal tubules and ovary of *Pygosteus pungitius* (9-spined stickleback).

*Effects*.—The following probably apply to this species, to *M. brevis*, and to *Chloromyxum elegans*:

Upon the kidney, Thélohan's observations are as follows:

The organ is often almost entirely invaded. Upon section one sees nearly all the tubes completely obstructed by psorospermic matter. The canaliculus invaded is dilated and attains relatively enormous proportions, the entire kidney being consequently enormously augmented in volume, and its function evidently must be almost completely abolished. A remarkable fact of this invasion of the renal canaliculi by the *Myxosporidia* is the small amount of disorder that they occasion. Beyond the dilatation of the tubes one observes only a little augmentation of volume of the nuclei of the epithelium. The cells are otherwise respected, and I have never seen the protoplasm of the myxosporidium invade them or insinuate itself between them. This is due without doubt to the dilatability of the renal tubules.

The following upon the ovary probably applies both to *M. medius* and to *M. brevis*:

Upon sections of this organ one sees the connective tissue invaded by the plasmic masses, which separate its fasciæ; certain invaded ovules have completely lost their normal aspect and present in their interior more or less confluent islets of psorospermic matter.

**72. *Myxobolus creplini* Gurley, 1893. Pl. 32, figs. 1, 2.**

(Psorosperms of *Acerina vulgaris*, Creplin, 1842, Wiegmann's Archiv. f. Naturgesch., 1842, I, pp. 61-3, pl. 1, figs. A-E; *ib.*, Rayer, 1843, Rayer's Archiv. de Méd. Comp., I, pp. 268-9; *ib.*, Dujardin, 1845, Hist. Nat. d. Helminthes, p. 644; "tailed" psorosperm of *Acerina* Leydig, 1851, Müller's Archiv., p. 222; psorosperm of *Acerina vulgaris* Leuckart, 1852, Archiv. f. physiolog. Heilkde, XI, p. 436, fig. 21e; *ib.*, Robin, 1853, Hist. Nat. de Végét. Parasites, pp. 312-14; spore of *Acerina vulgaris*, Weltner, 1892, Sitzgs-Ber. Ges. Naturf. Freunde, Berlin, 1892, pp. 29-31, 34).

*Myxobolus creplini*, Bull. U. S. Fish Com. for 1891, XI, p. 418; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.



Cyst not described; myxosporidium unknown.

*Spore*.—Perfectly transparent, colorless, much larger than any of Müller's species, body elongate, strongly ventricose-elliptic,  $17.3\mu$  long by  $5.8\mu$  broad; shell bivalve, of firm texture, enabling the spore to retain its shape on drying, splitting open after several days' immersion in water, the resulting median fissure extending nearly to the root of the tail; tail present, simple, diminishing in thickness from origin to its fine pointed extremity, about as long as or a little longer than the body (in 1 specimen  $2\frac{1}{2}$  times that length), often more or less deflected from the line of the antero-posterior axis of the body; contents of body cavity perfectly clear, granule-free, showing no trace of structure other than the capsules; capsules 2 (on transverse view only 1) of equal size, pale yellow, subcylindrical, situated at the anterior pole, diverging posteriorly or adnate to each other along their inner borders; in a single specimen beginning as a single cylindrical tube ( $\frac{1}{2}$  the length of the capsules), which divided posteriorly into the 2 capsules; the latter diverging from their origin to their blind posterior extremities (fig. d). Capsules become strongly wrinkled on drying.

*Habitat*.—On *Acerina cernua* L.; collected March 14, 1837.

**73. *Myxobolus strongylurus* Gurley, 1893. Pl. 31, fig. 5.**

(Psorosperms of *Synodontis schal*, Müller, 1841, Müller's Archiv., pp. 480-1, pl. 16, fig. 2; *ib.*, Müller, 1843, Rayer's Archiv. de Méd. Comp., I, pp. 222, 227, pl. 9, fig. 2; *ib.*, Robin, 1853, Hist. Nat. de Végét. Parasites, p. 295, pl. 14, fig. 4.)

*Myxobolus strongylurus*, Bull. U. S. Fish Com. for 1891, XI, p. 417; *Myxobolus strongylura* [error], Braun, 1894, Centralbl. Bakt. u. Parasitenkde, xv, p. 87.

*Cyst*.—Over 2.18 mm. ( $1'''$ ) in length.

Myxosporidium unknown.

*Spore*.—Body blunter anteriorly than in *M. schizurus*; length without tail  $9\mu$  ( $0.0040'''$ ); breadth,  $5.4\mu$ ; tail always undivided, very peculiar in being constantly oblique in the longitudinal plane, appearing straight when seen in transverse view; capsules, 2, of equal size. Spore sometimes showing at posterior end of capsule a dark punctule which occasionally causes a slight projection of the shell at this part.

*Habitat*.—Encysted in skin of cephalic region of *Synodontis schal* from the Nile.

**74. *Myxobolus monurus* Gurley, 1893. Pl. 32, figs. 3, 4.**

(Psorosperms of *Aphredoderus sayanus* Ryder, 1880, Amer. Nat., xiv, pp. 211-2, figs. 1, 2; parasite of *Aphredoderus savanus*<sup>1</sup> [error] Thélohan, 1892, Bull. Soc. philomat. Paris, iv, p. 177.)

*Myxobolus monurus*, Bull. U. S. Fish Com. for 1891, XI, p. 416; *ib.* of *Aphrododerus* [error] *sayanus* Braun, 1894, Centralbl. Bakt. u. Parasitenkde, xv, p. 87.

*Cyst*.—Lenticular, large, bulging, white, opaque, numerous (about 20 in the only fish seen), imbedded in the subcutaneous muscles, arranged as a rule in pairs on the opposite side of the body of the fish; membrane very thin; contents, a thick, white, creamy mass, containing multitudes of spores and of excessively minute round granules.

<sup>1</sup> "The parasite described by J. Ryder in *Aphredoderus savanus* constitutes probably a fourth species" [of Thélohan's genus *Henneguya*].

*Myxosporidium* unknown.

*Spore*.—Body lenticular or slightly obovate; tail present (rarely absent), thick at origin, attenuating gradually, more or less curved, between 2 and 3 times as long as the body, undivided; capsules, 2, of equal size, subparallel, on longitudinal view seen to be eccentric.

*Habitat*.—Encysted in subcutaneous intermuscular tissue of *Aphredoderus sayanus* Gilliams (pike perch), taken near Woodbury, N. J.

75. *Myxobolus macrurus* Gurley, 1893. Pl. 32, fig. 5; pl. 33, figs. 1-4.

(*Myxosporidia* of *Hybognathus nuchalis*, Evermann, 1892, Bull. U. S. Fish Com. for 1891, XI, p. 76).

*Myxobolus macrurus*, Bull. U. S. Fish Com. for 1891, XI, p. 416; *ib.* of *Hypognathus* [error] *nuchalis*, Braun, 1894, Centralbl. Bakt. u. Parasitenkunde, XV, p. 87.

*Cyst*.—Multiple (usually 15 to 20 or more), the size of a pin-head, sometimes separated, more frequently in contact, forming elongated masses 6 mm. by 2, or less, imbedded in the subcutaneous connective tissue; almost invariably situated upon some portion of the head. Out of a multitude of cysts upon more than 80 fish, I have seen but one exception, a cyst situated at the base of the pectoral fin, a few millimeters behind the head. The great majority of the cysts are concentrated in 2 lines along the 2 halves of the inferior maxilla between the bone and the skin.

*Myxosporidium* unknown.

*Spore*.—Tailed; body rounded-oblong, 10 or 11  $\mu$  long, 6 to 8  $\mu$  broad, 4  $\mu$  thick. Shell substance thin, colorless, perfectly transparent, very resistant to the strongest acids and alkalis, not stained by any of the reagents tried. Valves 2, superior and inferior, unequally convex. Superior valve with a very convex outer surface, to which corresponds internally a surface deeply concaved for the reception of the larger portion of the capsules and sporoplasm. Inferior valve outwardly convex-flattish, with a shallow line of depression across the middle portion of its external surface, to which corresponds on the internal surface a broad, gentle ridge, marking the space between the capsules and the sporoplasm. Ridge forming the anterior continuation of the tail, at the anterior extremity of the spore, projecting slightly in transverse view (optical section), as a blunt, nasute process.

Tail substance somewhat less transparent than that of the shell, completely dissolved by sulphuric acid (cold, concentrated) almost (usually entirely) invisible in balsam, the species then appearing untailed. Tail very long when complete (30 to 40  $\mu$  or less), the very attenuate posterior portion easily (and consequently frequently) broken off, the tail then appearing short, thick, and blunt. Tail consisting of a single long, posteriorly-directed median piece, and of two short, anteriorly-directed lateral pieces. Median piece, usually straight, frequently, however, more or less deflected to the right or left, or upward or downward, thick at its origin, attenuating gradually thence to the

acuate posterior extremity, destitute of apparent structure,<sup>1</sup> very liable to break off, the fracture always taking place evenly and never producing a ragged end. Lateral pieces 2, strongly curved, extending forward on either side from the anterior end of the median piece, applied closely to the rounded posterior portion of the shell about as far forward as the junction of the posterior and middle thirds of its outer margin; thickest at their origin, becoming very thin toward their anterior extremities. They have a slight expansion over the superior and inferior surfaces of the shell, thus tending to form a slightly cup-shaped receptacle for it. It is probable that they really extend forward along upon the surface and over the sides of the ridges, which structures appear as though continuous with them.

Capsules: 2, pyriform, somewhat diverging posteriorly, attenuated at the anterior end into the ducts which converge forward toward the median line, on either side of which they open. Capsular wall staining readily with and retaining tenaciously bismarck brown and fuchsin; rendered transparent by iodine water and by strong ammonia water. The filaments are thus seen lying coiled within the capsule. They appear not to stain with reagents which stain the walls, the capsule usually showing a lighter central and a darker circumferential portion. Relative to the occasional presence on or near the capsule of a dark "granule," see p. 220. The capsules are always surrounded by a clear space, the *pericystic*. This space never shows a double contour, never stains, and presents no appearance suggestive of an outer membrane. It is apparently a natural and presumably (by exclusion and analogy) a fluid-filled space. It does not stain with iodine, agreeing in this respect with the space (with which it is continuous) everywhere lining the inner surface of the shell, and differing in the same respect from the vacuolic space.

Sporoplasm: Inferior surface convex in all directions, showing a rounded postero-lateral margin,<sup>2</sup> extending from about the middle point of the lateral border of the spore on one side to the corresponding point on the opposite side. From these two points (infero-lateral cornua) the 2 antero-lateral borders curve inward and forward with a sharp anteriorly directed concavity to the median line where the sporoplasm is drawn out to a point (the infero-median cornu) which forms also the inferior extremity of a ridge shortly to be described as the supero-inferior intercornual ridge. The infero-median cornu is situated about at the level of the middle point of the antero-posterior diameter of the shell cavity. Lateral surface, extending forward for some distance

<sup>1</sup> Iodine (aqueous solution with potassium iodide) produces a decided beading of the median piece, transverse lines of division appearing, constituting a decided pseudo-segmentation. My attention was directed to this phenomenon by Dr. Stiles.

<sup>2</sup> Common, of course, to it and to the superior surface, being the line of intersection of the longitudinal plane with the interior surface of the shell.

convexly, both antero-posteriorly and supero-inferiorly, the cross-section of the sporoplasm at this point being unequally biconvex-lenticular. Anteriorly, however, each lateral surface is probably excavated for the lodgment of the posterior end of the capsule of the same side. The cross-section of the sporoplasm at the level of the infero-median cornu is a biconcavo-convex isosceles triangle. Superior surface convex in all directions with its postero-lateral margin coincident with the same margin of the inferior surface; differing from that surface mainly in the slighter concavity of the antero-lateral margins (and the consequently less mucronate shape of the supero-lateral cornua) and in the greater extension forward both of the supero-median and of the supero-lateral cornua. The supero-inferior intercornual ridge mentioned above curves (in the vertical plane) from the supero-median cornu downward and backward through the interior of the shell cavity to terminate in the infero-median cornu.

*Micro-chemistry.*—Hydrochloric acid alcohol carmine stains the nuclei better than other reagents. Iodine (aqueous solution with potassium iodide) stains the vacuole dark brown; stain removed by alcohol; staining most intense at first, the vacuole staining more rapidly than the sporoplasm. This reagent causes the separation of the tail from the body, and a beaded appearance of the tail. As, however, I have not detected this condition in other examination media, I suspect that it is not the normal structure. Finally iodine renders the capsular walls transparent and the filaments visible. Sulphuric acid (cold, concentrated) dissolves the tail (the shell remaining unaffected) and causes the valves to gape open, and finally to separate. Gently warmed, no further effect is produced. Heated to the boiling point, the valve substance is destroyed (dissolved?). Ammonia water renders the capsular walls transparent and the filaments visible. Balsam renders the tail invisible, the shell remaining visible.

*Habitat.*—Encysted on head of *Hybognathus nuchalis* Ag. (identification by Prof. B. W. Evermann), collected November 24, 1891, in the Neches River, 14 miles east of Palestine, Texas, by Prof. B. W. Evermann, U. S. Fish Commission. Water temperature 9.4° C. (49.5° F.). Disease very frequent.

*Effects.*—Although the tumors form quite extensive patches, the effect upon the fish could hardly, I think, be serious. That the movements of the jaw are not materially impaired is shown by the excellent nutrition of the fish. Indeed the present species seems rather a sub-commensal than a true parasite. Thélohan<sup>1</sup> reports that he saw a cyst shell out of its place in the tissue of the fish and fall into the water. Everything implies that a similar process takes place here, as superficial pitted scars were seen upon several specimens. These show no trace of long-continued ulceration, being very free from the puckering

<sup>1</sup> Annal. de Microgr., 1890, II, pp. 203-4.

thus caused. Moreover they conform very closely to the shape of the cysts. This is especially well shown where a cyst situated in the center of a group has shelled out, the surrounding cysts, preserving the shape of the cavity.

In this species, under influence of cold, concentrated sulphuric acid (which dissolves the tail) the valves separate, the divergence appearing always to begin at the posterior end. The appearances seem to favor the view that such divergence was the result of the previous solution of the tail, the 2 lateral pieces of which would thus act as a splint. As, however, examination of untailed species (in which I suspected the lateral pieces might exist without the median) failed to show evidence of the existence of the lateral pieces or even of the constancy of the initial posterior divergence, this function of the tail must be regarded as dubious. In any case, at least, one other causal factor must be involved in valve separation, as iodine, which produces separation of the tail, does not produce separation of the valves. I suspected that this might be exosmotic pressure from within, and attempted to produce valve separation by the action of strong glycerin used after iodine had detached the tail, but the results were indecisive.

This species is particularly interesting as exhibiting decided supero-inferior asymmetry, the superior valve being conspicuously more convex, and the supero-median cornu projecting farther forward. It is also important to note that the tail is not a shell process, but is, on the contrary, an independent structure with distinct optical and chemical characters.

**76. *Myxobolus* sp. incert.**

Psorosperms of *Coregonus fera*, Claparède, 1874, in Lunel's Hist. nat. des poissons d. bassin du Léman, p. 114.

*Cyst*.—A single one seen, 1 mm. in diameter; contents entirely different from those of the other branchial cysts, approximating to, without being perfectly identical with, those of the cysts of the muscles of the same fish.

Myxosporidium unknown.

*Spore*.—Distinguishable from those of the muscle cysts by their shorter and usually single tail, which, however, in a great number of individuals was bifurcate at the extremity.

*Habitat*.—Branchial arches of *Coregonus fera*.

**77. *Myxobolus* cf. *linearis*. Pl. 33, figs. 5-8.**

Cysts of base of dorsal fin of *Ancistrus melas*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, p. 417.

*Cyst*.—Subspherical, about 1 mm. in diameter, 7 in number in a row at the bases of the spines of the second dorsal fin.

Myxosporidium unknown.

*Spore*.—Body lanceolate; length of body, 19  $\mu$ ; breadth, 5 or 6  $\mu$ ; thickness, about 3  $\mu$ .

Shell consisting of 2 valves, superior and inferior in position; ridge present, forming continuation of tail. The tail in this species is a shell process, consisting of 2 halves, a superior and an inferior, each connected with and forming a solid process of the corresponding valve. Length of tail,  $38\mu$ . Valves separating very slowly in sulphuric acid (cold, concentrated), the gradual lateral shifting of one valve over another beginning within a few minutes and continuing for 20 or 30. Coincidentally the two tail halves diverge, serving well as indices of the amount of lateral shifting of the valves. Iodine fails to loosen the connection of the tail (or of either half) with the valves.

Capsules long, narrow, parallel-appressed; capsular index about 0.40; walls rendered transparent and filaments visible by iodine water.

Sporoplasm showing the usual anterior extension of the supero-median cornu. The other cornua are also recognizable. Vacuole present, subcircular in outline, usually placed toward the anterior end of the sporoplasm. As regards nuclei, hydrochloric acid alcohol carmine always stains as many as and usually 2, rarely 3; position inconstant, one or both being either before or behind the vacuole. In addition, there are constantly present, at or close to the extreme posterior end of the sporoplasm, 2 deeply stained dots, which are too minute to show any structural details.

*Habitat*.—7 or 8 cysts at bases of the spines of the second dorsal fin of *Ameiurus melas* Raf. (bullhead) from Storm Lake, Iowa, collected August 23, 1890, by Prof. Seth E. Meek, to whose kindness I am indebted for the specimen.

This species can only be compared with the next. The following summarizes Müller's scanty diagnosis of that form:

Body very narrow, 3 to 4 times as long as broad; capsules parallel-appressed; tail simple, occasionally double.

The present species answers to all of these characters, but they are too few to warrant the fusion of the two forms, although their identity may be strongly suspected. If established, their identity would constitute a very interesting fact, both in zoological and in geographical distribution, for we should then have a species found (so far) confined in its zoological range within the *Siluridae* and with a very wide geographical distribution.<sup>1</sup>

<sup>1</sup>For the geographical distribution (in South America) of *R. sebæ* and of *P. fasciatum*, see Eigenmann & Eigenmann, Revision So. Amer. Nematognathi (Occas. Papers Calif. Acad. Sci., San Franc., 1890), pp. 123, 209. Considering the names used by Müller, the date of his writing, etc., it seems rather probable that his localities were those known to Cuvier and Valenciennes (1840), viz, for *R. sebæ*, Surinam, Cayenne, Rio Janeiro, Buenos Ayres, and for *P. fasciatum*, Surinam.

**78. *Myxobolus linearis* Gurley, 1893. Pl. 36, fig. 2.**

(Psorosperm of *Pimelodus seba* and of *Platyostoma fasciatum*, Müller, 1841, Müller's Archiv., p. 489, pl. 16, fig. 10; *ib.*, Müller, 1843, Rayer's Arch. de Méd. comp., pp. 228-229, pl. 9, fig. 10; *ib.*, Robin, 1853, Hist. Nat. d. Végét. Parasites, p. 300, pl. 14, fig. 11.)

*Myxobolus linearis*, Bull. U. S. Fish Com. for 1891, XI, p. 417; *ib.* of *Pimelodes* [error] etc., Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

Cyst not described; myxosporidium unknown.

*Spore*.—Body very narrow; length, 3 to 4 times breadth; capsules parallel-appressed, in contact along their entire length; tail simple, occasionally double.

*Habitat*.—Cysts in membrane lining branchial cavity of *Rhamdia seba* Cuv. & Val.; cysts on branchial lamellæ of *Pseudoplatystoma fasciatum* L., from South American rivers.

**79. *Myxobolus schizurus* Gurley, 1893. Pl. 36, fig. 1.**

(Psorosperms of *Esox lucius*, Müller, 1841, Müller's Archiv., pp. 477-478, pl. 16, fig. 1; *ib.*, Müller, 1843, Rayer's Archiv. de Méd. Comp., I, pp. 219-222, pl. 9, fig. 1; *ib.*, Dujardin, 1845, Hist. Nat. des Helminthes, pp. 613, 614; *ib.*, Robin, 1853, Hist. Nat. des Végét. Parasites, p. 292, pl. 14, figs. 2, 3; *ib.*, Lieberkühn, 1854, Müller's Archiv., p. 5; ? *ib.*, Thélohan, 1890, Compt. Rend. hebdom Soc. Biol. Paris, II, p. 604; *ib.*, Weltner, 1892, Sitzgsber. Ges. Naturf. Freunde Berlin, pp. 29-35.)

*Myxobolus schizurus*, Bull. U. S. Fish Com. for 1891, XI, p. 417; *Myxobolus schiozurus* [error] Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Cyst*.<sup>1</sup>—Whitish, 0.44 to 1.09 mm. ( $\frac{1}{5}$ – $\frac{1}{2}$ ''') in diameter, membrane delicate, contents white, unaffected by water, consisting of finely granular matter and spores, the latter motionless.

Myxosporidium unknown.

*Spore*.—Body oval, double contoured, resembling and about the same size as the oval blood corpuscle of the fish; length 12  $\mu$  (0.0054'''), breadth 6  $\mu$  (7  $\mu$  Robin; 0.0026'''), thickness about one-half the breadth; border flattened-rotund, marked by a ridge which extends forwards on either side of the shell, projecting slightly in front; tail stout at origin, attenuating gradually, 3 to 4 times length of the body, not articulated, very frequently (probably as a rule) bifurcate at the tip, or for more or less of its length. Untailed forms very rare; capsules 2, of equal size, always diverging posteriorly; remainder of shell cavity filled with a transparent, rarely granular substance, differentiated by refraction from the shell substance.

*Habitat*.—Encysted in the orbit (never found elsewhere) in the cellular tissue of the eye-muscles, in the sclerotic, and between the last and the choroid of young *Lucius lucius* L. (pike) in May and June. Found in only about 10 per cent of the fish examined. Müller failed to find this disease in the North American pikes examined.

<sup>1</sup> These cysts are not to be confounded with similar white entozoan cysts. The latter are of more frequent occurrence in the orbit than the myxosporidian cysts. They are smaller in size (about 0.50 to 0.65 mm.) and have thick walls. Under the microscope the entozoan can be seen moving with transverse wrinklins of its cyst.

**80. *Myxobolus psorospermicus* Thélohan, 1892. Pl. 34.**

(Psorosperms of *Perca fluviatilis*, Bütschli, 1882, Bronn's Thier-Reich, I, pl. 38, fig. 16; *ib.*, Balbiani, 1883, Journ. de Microgr., VII, pp. 201, 203, fig. 42; psorosperms of *Esox lucius*, *ibid.*, pp. 201-2, fig. 41; *ib.*, Balbiani, 1884, Leçons sur les Sporozoaires, p. 132, fig. 37; psorosperms of *Perca fluviatilis*, *ibid.*, p. 133, fig. 38; *ib.*, Lankester, 1885, Ency. Britan., 9 ed., XIX, p. 855, fig. XVII, 43, 44; *ib.*, Thélohan, 1889, Compt. Rend. Acad. Sci. Paris, CIX, p. 604; *ib.*, Thélohan, 1890, Annal. de Microgr., II, pp. 202, 207, 211, figs. 5-7; *ib.*, Thélohan, 1890, Compt. Rend. hebdom. Soc. Biol. Paris, II, p. 604; tailed psorosperms of pike, *ibid.*; psorosperms of *Perca fluviatilis*, Pfeiffer, 1890, Die Protozoen als Krankheitserreger, 1 ed., p. 43; *ib.*, Pfeiffer, 1891, *ibid.*, 2 ed., p. 130.)

*Henneguya psorospermica*, <sup>1</sup> Bull. Soc. philomat. Paris, IV, pp. 167, 176.

*Myxobolus psorospermica* Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 418.

*Henneguya psorospermica* Braun, 1893, Centralbl. Bakt. u. Parasitenkde, XIV, p. 739.

*Myxobolus psorospermica*, Braun, 1894, Centralbl. Bakt. u. Parasitenkde, XV, p. 87.

Cyst and myxosporidium not described.

*Spore*.—Anterior extremity obtuse; length, 35 to 40  $\mu$  (Thélohan, 1892; 36  $\mu$ , Balbiani for spore of *Lucius*; 30  $\mu$ , Thélohan, 1890, for spore of *Perca*); breadth, 4  $\mu$  (Thélohan, 1890). Tail curved close against the body during development, becoming straight only after the rupture of the pansporoblast membrane; caudal index, 1. Capsules, 6 to 8  $\mu$  in length. Maximum number of nuclei, 3; vacuole present (Thélohan, 1890).

*Habitat*.—Branchiæ of *Lucius lucius* L. (pike) and of *Perca fluviatilis* (yellow perch).

*Remarks*.—In view of Thélohan's positive statement as to the identity of the forms habitant on the branchiæ of *L. lucius* and *P. fluviatilis*, I believe we are justified in referring all the forms figured to one species, although fig. 34 (pl. 4) differs somewhat from the rest.

**81. *Myxobolus kolesnikovi* Gurley, 1893. Pl. 35.**

(Psorosperms of *Coregonus*, Kolesnikoff, 1886, Vet. Vestnik Kharkoff, V, pp. 242-248, plate, figs. 1-3.)

*Myxobolus kolesnikovi* of *Coregonus fera* [error],<sup>2</sup> Bull. U. S. Fish Com. for 1891, XI, p. 417.

*Cyst*.—Numerous, sometimes as many as 80, length 10 to 30 mm., breadth 7 to 20 mm., spherical or oval, bean-shaped, yellowish-white, surface of cyst-wall smooth and shining, membrane of the thickness of a cigarette paper, rupturing by the slightest pressure of the forceps. Contents thick, yellowish-white, creamy, consisting of spores and an oily substance.

<sup>1</sup> "One finds on the branchiæ of the pike and of the perch a myxosporidian absolutely identical in the two cases and which it is certainly necessary to consider as constituting but a single species" (Thélohan.)

The words "Psorospermies de J. Müller" were evidently attached to this species inadvertently. Müller knew no species on the branchiæ of *L. lucius*. In this fish he observed them only in the orbit.

<sup>2</sup> Kolesnikoff does not mention any species.



*Myxosporidium*.—The following may refer to this stage. To me it is rather obscure:

Between the tailed spores were found in great numbers protoplasmic bodies of the size of a blood corpuscle or smaller, which were round and contained "semen" (?spores). The protoplasm of these bodies was seminal (?sporigenous). The nucleus was sharply defined and contained several semina (?granules).

*Spore*.—Round or oval with a sharp anterior end; shell double-contoured; substance homogeneous, texture reminding one of chitin, unaffected by acids and by alkaline hydrates; capsules 2, anteriorly placed; filaments gradually extruded under the influence of gentle heating. By means of staining with fuchsin or methylen blue performed after warming, there appeared in the spore a sharply defined "nucleus". Tail single or double, consisting of a substance similar to the shell, thick at its origin, attenuating gradually to its free extremity; shape similar to that of the tail of *M. psorospermicus* as figured by Bütschli.<sup>1</sup>

*Micro-chemistry*.—Fuchsin and methylen blue stain the spores and the extruded capsular filaments, but not the shell or the tail.

*Habitat*.—Cysts irregularly distributed in the interstitial connective tissue of the thoracic and intercostal muscles of *Coregonus*. Loosely united to the surrounding muscular tissue by spongy connective tissue and easily separable therefrom by its rupture.

As to the relation of this species to the next, see next page.

## 82. *Myxobolus* sp. incert.

Psorosperms of muscles of *Coregonus fera*, Claparède, 1874, in Lunel's Hist. nat. d. poissons du bassin du Léman, p. 113.

*Cyst*.—Five in number, varying in size from that of a filbert to that of a small walnut. Characters constant. Contents, a milky fluid or (from resorption of the more liquid portions) a caseous mass. This fluid or semifluid mass consists of psorosperms in great number, with a granular protoplasm between them.

*Myxosporidium*.—This granular protoplasm is without doubt the remains of the amœba at the expense of whose protoplasm, and within which, the psorosperms were formed. The protoplasm in fact contains "vacuoles" (pansporoblasts) which in the beginning are destitute of proper walls, but which form the point of departure for psorosperm production. The examination of one fragment of protoplasm is sufficient to show all transitions between the simple vacuoles (pansporoblasts) and the vesicles containing the 2 oval corpuscles [capsules] characteristic of the psorosperm, and a third corpuscle, whence will be derived the "blastema" (sporoplasm) which fills the posterior part of the body of the psorosperm. It is only a step from these vesicles to the imperfectly developed psorosperms disseminated through the protoplasm. These last already show all essential traits of the fully developed psorosperm

<sup>1</sup> Bronn's Thier-Reich, 1882, 1, pl. 38, fig. 16.

except that the 2 tails are still short and distant from each other at their origin. Besides they show an extreme transparency, their degree of refringency being very inferior to that of the psorosperm, thus easily escaping search in the midst of the very similarly refringent protoplasm.

*Spore*.—Characters constant; body lenticular; length, 8 to 10  $\mu$ ; tail not merely bifurcate, but double from the base, this feature, however, being only recognizable in a portion of the profile, as when the spore is seen from the face one tail exactly covers the other: capsules 2, ovoid.

*Habitat*.—Encysted in the muscles of *Coregonus fera*.

*Remarks*.—Very probably this form should be correlated with the preceding; but as Kolesnikoff has given no measurements and Claparède no figures, it is thought advisable to refrain from fusing them.

**83. *Myxobolus? diplurus* Gurley, 1893. Pl. 36, fig. 4.**

(Psorosperms from kidney of *Lota vulgaris*, Bütschli, 1882, Bronn's Thier-Reich, I, pl. 38, fig. 21; *ib.*, Lankester, 1885, Encycl. Britan., 9 ed., XIX, p. 855, fig. xvii, 42.)

*Myxobolus diplurus*, Bull. U. S. Fish Com. for 1891, XI, p. 418; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

No description. If Bütschli's figures are to be depended upon, this species is at once distinguished from all others of the genus by the posterior position of the capsules.

*Habitat*.—Kidney of *Lota lota* L. (ling).

Fam. CHLOROMYXIDÆ Gurley, 1893.

(“Chloromyxées,” et “Myxidées” (*pars*), Thélohan, 1892, Bull. Soc. philomat. Paris, IV, pp. 173, 176; Chloromyxea [Thél.] Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739.)

*Chloromyxide*, Bull. U. S. Fish Com. for 1891, XI, pp. 412, 418; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

*Definition*.—*Phanocystes* destitute of antero-posterior, but possessing bilateral symmetry;<sup>1</sup> capsules in 1 group at the anterior end; a bivalve shell, the plane of junction of whose valves is perpendicular to the longitudinal;<sup>2</sup> no vacuole; type genus *Chloromyxum*.

*Vacuole*.—Thélohan<sup>3</sup> is authority for the statement that this structure is absent from the sporoplasm of the *Chloromyxide* as here constituted. My observations on *C. (S.) ohlmacheri* confirm this.

*Pigment*.—Leydig (see p. 260) notes in the myxosporidium of *C. leydigii* a yellowish coloration which he attributed to bile-staining.

Mingazzini<sup>4</sup> also mentions this coloration, but does not comment upon its origin.

<sup>1</sup> Imperfect from unilateral position of sporoplasm in *Ceratomyxa*.

<sup>2</sup> An examination of *C. (S.) ohlmacheri* has confirmed the opinion hazarded in a former paper (Bull. U. S. Fish Com. for 1891, XI, p. 412), that in the *Chloromyxide* the valve-junction plane is the vertical.

<sup>3</sup> Bull. Soc. philomat. Paris, 1892, IV, p. 173.

<sup>4</sup> Boll. Soc. Nat. Napoli, 1890, IV, p. 160.

Finally Thélohan's observations on *Ceratomyxa sphaerulosa* (pp. 76, 277) indicate that perhaps a proper pigment (and not merely an extraneous one, as hæmatoidin) may exist in this genus.

# VI. CHLOROMYXUM Mingazzini, 1890.

Etymology not given.

Boll. Soc. Nat. Napoli, iv, p. 160; *ib.*, Thélohan, 1892, Bull. Soc. philomat. Paris, iv, pp. 173, 176; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, pp. 411, 412, 418; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, p. 739; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Definition*.—*Chloromyxidæ* with subspherical or ovate spores, whose breadth does not much exceed the length; valves hemispherical; sporoplasm bilaterally and symmetrically situated; type *C. leydigii*.

*Synonymy*.—By reference to table on page 115, it will be seen that *Sphaerospora* and *Myxosoma* differ in none of the characters there given, the genera at present resting solely upon spore-form. This is entirely insufficient to warrant the retention of both genera, especially as any reason which would justify the generic separation of the ovate from the subspherical bicapsulate spores, would equally justify a similar separation of the ovate from the subspherical quadricapsulate spores.

From *Chloromyxum* the *Sphaerospora-Myxosoma* section has indeed the additional character of 2 capsules as opposed to 4 in *Chloromyxum* proper. I have already given (p. 115) my reasons for regarding the number of the capsules as a character secondary in importance to their grouping and position. *Sphaerospora* (including *Myxosoma*) is therefore here accorded subgeneric rank.

## CHLOROMYXUM, *sens. strict.*

*Definition*.—Quadricapsulate *Chloromyxa*; type *C. leydigii*.

93. *Chloromyxum incisum* Gurley, 1893.<sup>1</sup> Pl. 37, fig. 1.

(*Psorosperms* of *Raja batis*, Leydig, Müller's Archiv., 1851, pp. 225-6, 234, pl. 8, fig. 4a-f.)

*Chloromyxum incisum*, Bull. U. S. Fish Com. for 1891, xi, p. 419; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

Cyst unknown.

*Myxosporidium*.—Biliary-yellow, mostly roundish or somewhat elongate, 29 to 88  $\mu$  (.0135-.0405''') in diameter, without or with 1 to 4 pansporoblasts (*Tochterblase*), most of which last contain spores. As in the spore of *Squatina squatina* (*M. leydigii*), the sporoblasts increase at the expense of the other portions of the cell contents until they nearly fill the cell (fig. 1e, f).

*Spore*.—Sharply cuneate-ovate, posterior border radiate-incised (causing it to resemble a radiate-ribbed Lamellibranch shell); capsules 4, situate anteriorly, converging.

*Habitat*.—Free in gall bladder of *Raja batis* L. (skate); present in great numbers.

<sup>1</sup> Concerning the relation between this species and the next, see the latter, under *Synonymy*.

94. *Chloromyxum leydigii* Mingazzini, 1890. Pl. 37, figs. 2-7; pl. 38; pl. 39, figs. 1-3.

<i>Squatina angelus</i> , "psorosperms" of.	<i>Spinax vulgaris</i> "psorosperms" of.	<i>Torpedo narke</i> , "psorosperms" of.	<i>Scyllium canicula</i> , "psorosperms" of.	leydigii.	plagiostomi.	Date.	Authority; reference.
× 1	× 2	× 3	× 4	-----	-----	1851	Leydig, Müller's Archiv., pp. 224-5, 233-4, pl. 8, figs. 1-3, 5.
-----	-----	-----	-----	-----	-----	1852	Leuckart, Archiv. f. physiol. Heilkde, XI, p. 435, plate, fig. 22. <sup>5</sup>
-----	-----	-----	-----	Chloromyxum.	-----	1890	Mingazzini, Boll. Soc. Nat. Napoli, IV, pp. 160-4.
-----	-----	-----	-----	-----	Myxosporidium.	1891	Perugia, Boll. Scientif., Pavia, XIII, p. 23, figs. 1-6.
-----	-----	-----	-----	Chloromyxum.	Myxosporidium.	1892	Thélohan, Bull. Soc. philomat. Paris, IV, pp. 166, 169, 170, 173, 176.
-----	-----	-----	-----	Chloromyxum.	-----	1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, pp. 418-19.
-----	-----	-----	-----	Chloromyxum.	-----	1893	Braun, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 738-9.
-----	-----	-----	-----	Chloromyxum.	-----	1894	Braun, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

<sup>1</sup> Leydig's description is as follows (p. 233, pl. 8, fig. 1a-f): *Myxosporidium* (developmental stages). (1) Roundish myxosporidia (*Mutterblase*),  $29\mu$  to  $118\mu$  ( $\cdot 0135$  to  $\cdot 0540''$ ) with a thin membrane and yellowish semifluid contents, containing a mass of yellow granules concentrated toward the center, leaving a granule-free border (fig. 1a). (2) Other myxosporidia of the same size contain, in addition, several transparent pansporoblasts (*Tochterblase*), whose number varies with the size of the myxosporidium, the smaller having but 1, the largest as many as 6. (3) Other myxosporidia show spores in the sporoblasts, always 1 in each (fig. 1c, d). (4) In the later stages the sporoblasts become very large, nearly filling the myxosporidium, and separated from its membrane only by a zone which represents a greatly diminished state of the granular mass. Yellow color due to the absorption of bile pigment. That the pansporoblast membrane is impervious to this pigment is shown by the unstained condition of the latter. *Spore*: Sharp-contoured, untailed, acute cuneate-oval, anterior extremity pointed. Capsules 4, situated at the anterior end. Free spores also occur. *Habitat*: Free in gall-bladder of *Squatina angelus*.

<sup>2</sup> The form found in gall-bladder of *Acanthias* (*Spinax*) *vulgaris* is (fide Perugia) referable to this species. Leydig's description is as follows (pp. 224-5, 233, pl. 8, fig. 2): *Myxosporidium*: Visible to naked eye, similar to that of *Squatina angelus* except that the appearance is more varied; round, vermiform, and retort-shaped forms occurring; frequently 2 or 3 round forms are united resembling a segmenting ovum; no movements or pansporoblasts seen. *Habitat*: Free in gall-bladder of *Spinax vulgaris*.

<sup>3</sup> Leydig's description (pp. 225, 233, pl. 8, fig. 3): *Myxosporidium* (developmental stages). (1) Large ( $29$  to  $118\mu$ ;  $\cdot 0135$  to  $\cdot 0540''$ ) yellow club-shaped protoplasmic masses of same general character as in *Squatina angelus*; pansporoblasts absent from this stage. (2) The large yellow masses contain much smaller ( $15\mu$ ;  $\cdot 00675''$ ) colorless vesicles with granular contents, the latter mostly heaped together. (3) A transparent pansporoblast is visible through the finely granular contents. On addition of sodium hydrate, spores become visible in it. Numerous free spores are also seen. *Habitat*: Free in gall-bladder of *Torpedo narke*.

<sup>4</sup> Leydig's description (pp. 225, 234, pl. 8, fig. 5): *Myxosporidium*: Size  $29\mu$  to  $147\mu$  ( $\cdot 0135$  to  $\cdot 0675''$ ); shape, roundish, elongated, retort-shaped, or vermiform with clubbed ends. Many show only membrane and contents; others show well-developed pansporoblasts, sometimes as many as 12, each containing 1 spore. *Habitat*: Free in gall-bladder of *Scyllium canicula*.

<sup>5</sup> On the page cited, Leuckart virtually says that his figure is "after Leydig," and a comparison with figs. 2a<sub>1</sub>, 2a<sub>2</sub> (plate 39) shows it to be a generalized composite from them.

### Concerning the synonymy, Mingazzini says:

All those examined by me in the various species of the *Plagiostomi* (*Torpedo*, *Scyllium*, *Squatina*, *Trygon*, *Raja*, *Mustelus*, *Pristiurus*, etc.) belong to the same species.

There is, however, in Mingazzini's paper almost nothing to show that he studied the spore at all. Only a single sentence refers to the

structure of the spore, viz, "Its theca shows an oblique striation in two contrary directions." Moreover, he unfortunately fails to indicate the species of fishes which he examined.<sup>1</sup>

Perugia, however, has given a list of the species of fishes he examined, which includes 2 species investigated by Leydig. He says:

While Leydig had observed that certain spores were striated and others not, Mingazzini says that the striæ are common to all, and is of opinion that there is question of but a single species, an opinion which I believe to be correct.

In describing *Chloromyxum leydigii*, Thélohan<sup>2</sup> says it has

Great striæ upon the shell, which, in passing round the posterior part of the spore, give it a toothed appearance.

It is thus evident that he includes with the present species *C. incisum*. As there is nothing, however, anywhere in the literature to show that he himself ever studied the spores of *C. incisum*, it is very probable that this statement is only intended as representing the consensus of opinion, that is, Mingazzini's and Perugia's views.

As regards Mingazzini's, we have (1) no evidence that he ever examined the gall bladder of *Raja batis*, and (2) only the very loose statement given above (which practically amounts to nothing), so that his opinion that there is but one species is a mere dictum, and even that does not necessarily, as far as the record shows, refer distinctly to this case.

Further, although Perugia notes the discrepancy between Leydig's and Mingazzini's observations and ranges himself with Mingazzini, it appears that he did not examine the gall bladder of *Raja batis*, and the general statement that "the striæ are common to all" seems to me too vague to warrant the fusion of 2 such distinct spore-forms as those here separated as *Chloromyxum leydigii* and *C. incisum*. Until distinct and detailed comparisons between the spores habitant in the gall bladder of *Raja batis* and those habitant in the gall bladders of the other Plagiostomes shall have been made and properly recorded, the specific identity of the 2 forms can not be admitted.

*Myxosporidium*.<sup>3</sup>—Examined in the bile they have the form of true plasmodes, consisting of a diversely ramified, yellow globular protoplasm, movements exceedingly slow. A few minutes after being placed on the slide they suddenly undergo modification, throwing out an external layer of colorless refracting protoplasm, which (especially at the extremities of the individual) suddenly protrudes filiform thin pseudopodia, which soon become more robust. They also modify their

<sup>1</sup> In this connection the following judicious criticism of Perugia's upon Mingazzini's work may be quoted: "He had an opportunity to make interesting observations, but he might well have set them forth in greater detail in his paper, especially as regards the various phases of formation of the spore, which he affirms he observed taking place in the vacuoles designated by Leydig as daughter-cells" [pan-sporoblasts].

<sup>2</sup> Bull. Soc. philomat. Paris, 1892, IV, p. 176.

<sup>3</sup> Description, Mingazzini's.

form, becoming globular or more or less ellipsoidal. It is important to note that in some individuals the entire protoplasm is transformed, changing from globular and yellow to spongy and colorless, the several globules disappearing almost in an instant, changing directly into clear protoplasm, not growing smaller, as might be thought. This shows how rapidly the protoplasm may change its constitution. Nucleus not found either in fresh material or in that treated by hydrochloric or acetic acid. Anilin stains only show here and there deeper colored granules, which, however, could not have the signification of nuclei.

Relative to the nuclei, Thélohan, however, says:

In the myxosporidium of *Chloromyxum leydigii*, as in the other forms, I have been able to prove the presence of numerous nuclei; they are, indeed, of rather small size, but nevertheless are easily recognized in sections, and if, as is probable, Mingazzini did not observe them, he did not have recourse to this method.

"*Gregarinoid forms.*"—In some gall bladders of the plagiostomes, Mingazzini found in summer also other forms of a very different figure, which were often united to the myxomycetous forms. These forms were uniformly cylindric-elongate, with one end obtusely rounded and the other drawn out to a sharp point in the form of a long tail four or five times as long as the body, sometimes multiple. Size varying greatly; no very small ones seen; large ones equaling the size of adult myxosporidians. Movements rather rapid, always taking place blunt end foremost. Protoplasm hyaline, or showing round hyaline globules arranged in regular longitudinal rows. Many contain a subcentral nucleus. Anteriorly the protoplasm contains rather numerous small, strongly refracting granules. This form thus resembles a monocystid Gregarine, but possesses peculiarities which differentiate it therefrom. For, first, an external membrane is wanting, as shown by negative microscopic investigation and by the protrusion (in individuals kept for many hours on the slide) from the blunt end of thin pseudopodia, which bear a great resemblance to those emitted under the same conditions by the *Myxosporidia*; and, second, no known monocystid possesses such a whip-like tail. Besides these forms others occur, which, while resembling in figure the preceding, have their protoplasm more or less charged with yellow granules resembling those of the adult *Myxosporidia*. Between these and the *Myxosporidia* are found other forms departing for the most part by more profound alterations of form from the first ones. Further, the more advanced gregarinoid forms, which possess refracting hyaline globules, take on the character of more adult forms, transforming their hyaline globules into yellow globules. From what precedes we thus see that the *gregarinoid forms are phases in the development of the myxosporidia of the plagiostomes* [italics his own].

Commenting upon this view, after noting that Mingazzini remarked that these views of the development of the *Myxosporidia* (i. e., via the "gregarinoid forms") did not accord with those held by Lieberkühn and Balbiani, Perugia<sup>1</sup> says that his own observation of the exit of the

<sup>1</sup> Boll. Scientif., Pavia, 1890, XII, pp. 138, 139.

amœboid sporoplasm from the spore (see below) causes him to support the opinions of Lieberkühn and Balbiani. Unfortunately, however, he adds the following:

Finally, also, the observations of Thélohan upon the failure of the filaments in the capsules of many spores is not favorable to the mode of view of Mingazzini.

Here again we have the *ribbonettes* and the *capsular filaments* confounded, another instructive warning against the application of the same name to two entirely different structures (see also p. 87).

Perugia further remarks (p. 138) that if the "gregarinoid forms" be regarded as larval stages the adult forms represent a retrogression, inasmuch as the "gregarinoids" with a nucleus and the protoplasm regularly disposed, need only a cuticle to be monocystids, while the adult stages, destitute of a nucleus and with the protoplasm never regularly disposed, are much farther removed therefrom. Perugia was, however, unable to find any such "gregarinoid forms."

Kruse, however, says:

Very interesting is an observation of Mingazzini's, which the author can confirm. In the gall bladder of the Selachians are found, besides typical *Myxosporidia*, long-drawn-out, tailed bodies, which move in Gregarine fashion, but which, on the other hand, are connected by manifold transitions with the amœboid forms.

*Spore formation.*—Rapidity of spore formation is truly extraordinary, most of the individuals having spores formed or in course of formation in less than 15 minutes. At undetermined points in the endoplasm (in the middle or near the periphery) appear round vacuoles of clear protoplasm, which, like the ectoplasm, originate by a rapid transformation of the yellow protoplasm. This vacuole presently acquires an enveloping membrane, and within it is formed the spore. Its theca shows an oblique striation in two directions. Spores may arise in individuals whose protoplasm is little modified, i. e., almost entirely composed of yellow granules, the spores being then inclosed in a membrane, round in form, formed from the yellow protoplasm, and containing also a colorless refracting liquid; or the spores may form in colorless protoplasm, in this case without the enveloping membrane, the spores issuing free and floating in the bile. Where, as sometimes happens in the first case, spores form at the periphery, they form, in growing, a sort of crown around the individual, and the spore is not set free until the enveloping membrane is well formed (Mingazzini).

Normally the pansporoblast shows at some portion of its circumference a distinctly semilunar aggregation of protoplasmic granules. Under the influence of reagents (e. g., osmic and sulphuric acids) the pansporoblast membrane bursts, discharging its contents, and remaining as a hyaline empty sac (Perugia).

*Spore.*—Untailed; cuneate-ovate; capsules 4. Perugia saw the exit of the sporoplasm from a spore of the gall bladder of *T. narke*. The large striae on the shell render the posterior border of the shell in contour dentate (Thélohan, 1892; see also p. 261).

Table of hosts.

Leydig.*	Mingazzini.†	Perugia.	Latest synonymy by Dr. Theodore Gill.	Common names.
Seyllium canicula.	Mustelus	Mustelus laevis	Galeus sp. Galeus mustelus	Smooth dog-fish. Large-spotted dogfish.
	Scyllium	Scyllium stellare	Scylliorhinus sp. Scylliorhinus stellaris	
Spinax vulgaris	Pristiurus	Acanthias vulgaris	Pristiurus melanostomus Bon	Spiny dogfish.
Squatina angelus	Squatina		Squatina squatina L.	Angel-fish.
Torpedo narke	Torpedo	Torpedo narce	Squatina sp. Torpedo torpedo Gmel.	Electric ray.
	Torpedo	Torpedo marmorata	Torpedo sp. Torpedo marmorata	
[Raja batis ‡]	Raja	Raja clavata	Raja sp. Raja clavata	Skate. Thornback.
	Trygon	Myliobatis aquila	Dasyatis sp. Cephaloetherus aquila	Stingray. Eagle ray.

\* By an evident misprint (*rinnenne* instead of *rinnenni*; "he found" instead of "I found") Perugia (Poll. Scientif., Pavia, 1890, XII, p. 136) states that Leydig, instead of Perugia himself, found this form in the series of hosts examined by Perugia.

† Mingazzini gives nothing but the generic name of the host. As there is nothing to indicate the identity of the species of hosts with those examined by the other authors, they are noted separately.

‡ This species I regard as distinct (see p. 261).

**95. Chloromyxum fluviatile** Thélohan, 1892. Pl. 39, fig. 4.

Bull. Soc. philomat. Paris, IV, pp. 173, 176, fig. 2; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 418; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 738, 739; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

Cyst none.

*Myxosporidium*.—The ectoplasm emits lobed pseudopodiae. Endoplasm, when young, colorless; when older, yellow; color appearing not to be located in special spheres.

*Spore formation*.—Number of spores formed in each myxosporidium indefinite.

*Spore*.—Nearly regularly spherical; size about 5 to 7  $\mu$ ; shell bivalve; bearing small, often difficultly visible, spines; ridge present; capsules 4; sporoplasm nonvacuolate.

*Habitat*.—Gall bladder of *Leuciscus* (*Squalius*) *cephalus* L.

This species is apparently rather rare; seen only twice; it is nearly related to *C. leydigii* (Thélohan).

**96. Chloromyxum mucronatum** Gurley, 1893. Pl. 39, figs. 5, 6.

(Psorosperm of *Gadus lota* Lieberkühn, 1854, Müller's Archiv., pp. 352-3, 368, pl. 14, figs. 5, 6; *ib.*, Lieberkühn, 1854, Bull. Acad. Roy. Belg., XXI, pt. 2, p. 22, name only; *ib.*, Leuckart, 1879, Parasiten des Menschen, 2 ed., p. 248, fig. 99a; *ib.*, Bütschli, 1882, Bronn's Thier-Reich, I, pl. 38, fig. 17; *ib.*, Balbiani, 1883, Journ. de Microgr., VII, pp. 201, 203, fig. 45; *ib.*, Balbiani, 1884, Léçons sur les Sporozoaires, pp. 130, 133, fig. 41; <sup>1</sup> *ib.*, Leuckart, 1886, Parasites of Man, 2 ed., p. 197, fig. 99a; *ib.*, Koch, 1887, Encyclop. d. gesamt. Thierheilkde u. Thierzucht, IV, p. 94, fig. 668, 3.)

*Chloromyxum mucronatum*, Bull. U. S. Fish. Com. for 1891, XI, p. 419; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

<sup>1</sup> See also below, under *C. elegans* (p. 266).



*Myxosporidium*.—The largest attaining  $75\ \mu$  ( $\frac{1}{50}'''$  Lieberkühn), the smallest the size of a blood corpuscle; spherical or ellipsoidal, more rarely irregular, membraneless, containing irregularly scattered fat-like globules.

*Spore formation*.—Many myxosporidia appear destitute of fat granules, but show a large number of structureless gelatinous globules; other myxosporidia show partly the same globules, partly similar ones of the same size containing 4 capsules whose apices are approximated. Many globules show only faint indications of such capsules. Sometimes 2 such globules occur inclosed within a common structureless membrane. Besides these, developed psorosperms occur, both individually and in heaps, held together by a mucoid substance.

*Spore*.—Sharp-contoured, subglobular, mucronate anteriorly; length *ad max.*,  $8\ \mu$ ; capsules 4, converging anteriorly.

*Habitat*.—Free in urinary bladder of *Lota lota* L. (ling). Found in about 20 per cent of the fishes examined.

*Remarks*.—Lieberkühn emphasizes the striking resemblance between this species and those described by Leydig from the gall-bladder of the Plagiostomes (*Chloromyxum leydigii* and *C. incisum*). He notes, however, that *C. mucronatum* differs from Leydig's forms in the absence of a membrane around the myxosporidium, and in the absence of the pansporoblastic vesicles (Leydig's *Tochterblase*). From later researches it is easy to interpret Lieberkühn's results in harmony with those of Leydig, as the vesicle stage of the pansporoblast is merely a later stage of the gelatinous globules of the above description (see pp. 81, 286).

#### SUBGEN. SPHEROSPORA Thélohan, 1892.

Etymology not given.

Bull. Soc. philomat. Paris, iv, p. 175; *Myxosoma* et *Mixosoma*<sup>1</sup>, *ibid.*, p. 175; subgen. (including *Myxosoma* and *Mixosoma*) of *Chloromyxum*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, pp. 411-412, 418-419; *Sphaerospora* et *Myxosoma*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, p. 739; *ib.*, Braun, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Definition*.—Bicapsulate *Chloromyxa*; type *Chloromyxum* (*S.*) *elegans*.

*Species*.—The study which, through the kindness of Dr. Ohlmacher, I was able to make of *C. (S.) ohlmacheri* enabled me to recognize 2 other species in the literature which should be referred to this subgenus. The first is Balbiani's spore of *Acerina cernua*, which I have named *Myxobolus perlatus*. The median anterior and posterior mucronate projections and the median line shown in Balbiani's figures, can be respectively interpreted only as the ends and the intervening portion of the ridge. In other words, the valve-junction plane is vertical. The appearances are identical with those shown by *C. ohlmacheri*. The second is Bütschli's spore of the ovary of *Lota lota*. Though Bütschli's figures represent it as bicapsulate it should be compared with *C. mucronatum*.

<sup>1</sup>Type *Myxosoma dujardini*.

88. *Chloromyxum* (*Sphærospora*) *elegans* Thélohan, 1892. Pl. 40, fig. 1.

(Myxosporidian spores of *Gasterosteus aculeatus* and *G. pungitius* (pars), Thélohan, 1890, *Annal. de Microgr.*, II, pp. 193, 200, 203, 209, pl. 1, fig. 1.)

*Sphærospora elegans*, Bull. Soc. philomat. Paris, IV, pp. 167, 175.

*Chloromyxum elegans*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 419.

*Sphærospora elegans*, Braun, 1893, *Centralbl. Bakt. u. Parasitenkde*, XIV, p. 739.

*Chloromyxum elegans*, Braun, 1894, *Centralbl. Bakt. u. Parasitenkde*, XV, p. 87.

*Synonymy*.—In 1890 Thélohan described the present species and *M. medius* as spores occurring in the renal tubules of *G. aculeatus* and *P. pungitius*. He remarked that the 2 entirely different forms of spore are found in close association, occurring not only in the same kidney, but side by side in the same tube of the kidney. Their relation to each other could not be determined, as he was unable to trace them back to the myxosporidium.

M. Thélohan writes me (1893) that:

In putting an interrogation point in regard to the presence of *Sphærospora elegans* in the kidney of *Lota lota*, I had in mind Balbiani's fig. 41. The spores which that figure represents are indeed a little less regularly spherical than those of *Sphærospora* and present a more pronouncedly attenuated extremity. Not having observed *Myxosporidia* in the *Lotas* that I have been able to examine, I do not know whether these fish contain exactly the same species as *G. aculeatus*. The figures of Lieberkühn (*Müller's Archiv.*, 1851, pl. 14, figs. 5, 6) certainly do not belong to *Sphærospora*. They, in fact, present 4 polar capsules, and are rather near *Chloromyxum fluviatile*. Still they form, I believe, a distinct species.

A close study of these figures has led me to doubt seriously whether Balbiani's fig. 41 can be correlated with *Chloromyxum* (*Sphærospora*) *elegans*. The whole question hinges upon the number of capsules in Balbiani's spore. The close similarity between his figure and Lieberkühn's fig. 6, the fact that quadricapsulate forms have frequently been figured by the authors as bicapsulate, and finally the close approximation in habitat (kidney and urinary bladder of same fish<sup>1</sup>), all point toward the synonymy given above.

Cyst none; myxosporidium unknown.

*Spore*.—Round, nearly spherical, untailed, 8 to 10  $\mu$  (Thélohan, 1892; 9 to 12  $\mu$ , *ibid.*, 1890). Ridge present, terminating in a slight projection at each end of the spore.

*Habitat*.—Almost constantly present in the renal tubules of *Gasterosteus aculeatus* (stickleback) and those of *Pygosteus pungitius* (9-spined stickleback); ? also in kidney of *Lota lota*<sup>2</sup> (ling); "accidentally" present in kidney of *Phoxinus phoxinus* L., ovary of *G. aculeatus* and that of *P. pungitius* (all *vide* Thélohan; the last two in a letter to the author, 1893).

*Effects*.—See p. 248.

<sup>1</sup> Balbiani does not give the seat. Thélohan cites it as the kidney (*vide* specimens in Collège de France?).

<sup>2</sup> The form habitant here I have referred to *Chloromyxum mucronatum* (see that species, and the paragraph above in this one).

89. *Chloromyxum* (*Sphærospora*) *ohlmacheri* Gurley, 1893. Pl. 40, fig. 8; pl. 41, figs. 1-3.

(*Myxosporidia* of *Bufo lentiginosus* Shaw, Ohlmacher, 1893, Journ. Amer. Med. Assoc., XX, pp. 561-7, plate, figs. 1-4.)

*Chloromyxum ohlmacheri*, in Whinery, N. Y. Med. Journ., LVIII, pp. 660-662, figure.

Cyst unknown.

*Myxosporidium*.—No myxosporidium could be detected. From this Ohlmacher concludes that:

It is probable that, in this case, the parasite did not reach its adult condition in its batrachian host, but here only passed one stage of its existence, that is, the spore stage.

*Spore*.—Transversely elliptic, about  $6\ \mu$  long and  $8\ \mu$  broad. Shell bivalve, valve-junction plane perpendicular to the longer axis of the spore; staining with gentian violet (Gram's method); exhibiting a well-defined undulate-parallel longitudinal striation, the optical expression of the spiral-coil structure of the shell. Ridge present, marking the line of junction of the valves. No loosened band (apparently springing, like a loosened barrel hoop, from the uniting edges of the spore-valves), such as Lutz describes, could be demonstrated.

Relative to the arrangement of the spore contents, Ohlmacher says:

On the side of the pole corpuscles opposite the plasmatic body the vacuole occurred. This space was unstained in specimens in which the excess of stain had been washed out; but in overstained spores the vacuole retained the dye, though not so strongly as the pole corpuscles and the plasmatic body.

Interpreted in connection with the orientation of the spore, this may be construed to mean that the contents of the shell cavity consist (from before backward), first, of a clear, nonstaining space (part of the pericystic space, and of course not to be confounded with the vacuole, which is intra-sporoplasmic); next, the capsules, and last (and most posterior), the sporoplasm.<sup>1</sup>

Capsules: Lying side by side, 2, occasionally only 1, a condition explicable, at least in part, Ohlmacher thinks, as spore mutilation in the technique; length, 3 to  $3.5\ \mu$ ; staining bright red, but showing no evidence of structure with Pfitzner's alcoholic safranin. Relative to their position, Ohlmacher remarks that—

The situation of these polar corpuscles on the side of the spore is peculiar, and in this respect our myxosporidia differ from those thus far described.

As shown below, this view is due to a nonorientation of the spore.

In safranin preparations the bright red capsules were frequently observed outside of the spores in the tissue of the kidney. Whether these extra-sporal capsules had migrated during life or had been displaced by the technique, it is, Ohlmacher says, impossible to assert positively. He continues:

I am of the opinion, however, that the migration of the pole corpuscles is a natural phenomenon in these organisms, and that it has as much or more weight in the life

<sup>1</sup> Subsequent examination of the spore confirmed this orientation.

history than the migration of the plasmatic mass usually described. The presence of many empty capsules<sup>1</sup> in the sections would lend weight to this view of the expulsion of the contents of the spore, and in fig. 4a I have represented a capsule<sup>1</sup> with a single pole corpusele, which appeared to be in the act of escaping through a rent in the capsule.

Filaments best seen in sections, stained with Babes's anilin-water safranin where they stain prominently yellow; length varying considerably, many occurring curled up at the end as though only partly unwound, measuring when fully projected 6 to 8 times the spore-breadth, extending far into the surrounding tissues; sometimes dimly visible through capsular wall; extruded parallel to the shorter (antero-posterior) diameter of the spore.

Sporoplasm varying considerably in size and shape, and sometimes filling all the extra-capsular portion of the shell cavity; in this condition presenting no evidence of segmentation. In other cases less extensive, being sometimes very small and shrunken,<sup>2</sup> the sporoplasm then frequently showing a well-defined segmentation, the line of division extending through its middle [i. e., coinciding with the vertical plane]. Each sporoplasm-half envelops, in the form of a well-defined crescent, the corresponding capsule. Nonvacuolate (letter to author, 1893).

The sporoplasm stains with Pfützner's alcoholic safranin a light pinkish hue, appearing under a Leitz  $\frac{1}{12}$  in anilin-stained sections, delicately granular; no other structure discernible. Nucleus and evidence of nuclear contents invariably absent. Ohlmacher adds:

I could not even demonstrate the micrococci-like particles in the plasmatic body, as have been described by Lutz, or the safranophile particles of Bütschli.

Micro-chemistry: Ohlmacher finds the sporoplasm constantly cyanophilous, the capsules constantly erythrophilous. This occurs with carbolic fuchsin and carbolic iodine green (Russell's method); the capsules staining a brilliant red, the sporoplasm light green. The tint of the sporoplasm (consequently also the degree of dichromophilism) varies from violet to a well-defined green. This difference depends in large part on the developmental stage of the sporoplasm. Where large and unsegmented and occupying a large part of the shell cavity the green stain was less clearly defined; where more condensed and divided into the 2 crescents closely applied to the capsules, the green was well marked. A striking differentiation is produced by Pfützner's alcoholic safranin, followed by aqueous methyl blue, rapid washing in alcohol, and clearing in xylol. The Biondi-Heidenhain triple stain and Watasé's cyanin-chromatrop failed, a result attributed to nonpenetration of the shell by the stain. On the other hand, the success of fuchsin-iodine-green and safranin-methyl-blue seems, Ohlmacher says, to be due solely to their more powerful staining properties, which permit them to penetrate the somewhat resistant shell.

This dichromophilism of the capsule and sporoplasm Ohlmacher com-

<sup>1</sup> By this term he means the spore-shell.

<sup>2</sup> Due, I think, to absolute alcohol fixation.

pare with the observations of Auerbach and others,<sup>1</sup> but without affirming Auerbach's interpretation of dichromophilism as indicative of nuclear bisexuality.

*Habitat*.—Host: *Bufo lentiginosus* Shaw (a toad). The single specimen was a large female, sent with a lot of frogs (which latter showed no unusual mortality) from the country to the laboratory early in September. A gradual increase in size took place in the toad and finally became particularly noticeable, but this was unconsciously ascribed to development of ova. About November 15 the specimen was noticed lying on its back, apparently dead, showing on careful examination, however, a faint flutter of the pleural wall over the heart, but no respiration.

Dr. Ohlmacher has kindly informed me (letter, 1893) that the locality whence all the specimens were obtained is Sycamore, De Kalb County, Illinois. Three more specimens of *B. lentiginosus* collected there July, 1893, showed the same myxosporidian species, but not in such numbers. All of the toads thus far examined have been females. (Later the same condition was found in the males.)

Seat: Almost invariably present in larger or smaller groups in the lumen of the urinary tubules; never within the epithelial cells, which latter never show the nuclear metamorphosis occurring with the intracellular *Sporozoa*; occasionally found in sections among the blood corpuscles in the large blood vessels, it being here impossible to say that it might not have been due to displacement during the technique; never found in the glomeruli; occurring sparingly in the collapsed folds of the urinary bladder, always on the bladder surface, never imbedded in the bladder wall; also free in the urine.

*Microscopic technique*.—Fixation by absolute alcohol or Flemming; imbedding in xylol-paraffin; affixing by the water-albumen method; staining with various anilins.

*Mode of infection*.—As to the origin of the myxosporidian infection, it can only be conjectured, Ohlmacher says, that it must have occurred by way of the cloaca to the bladder, and from here the parasites ascended the urinary passages. It is probable that in this case the parasite did not reach its adult condition in its batrachian host, but here only passed one stage of its development, the spore stage.

*Pathology*.—Abdomen containing a large quantity of straw-colored, serous fluid derived from the abdominal cavity and the subcutaneous lymph sinuses; to this fluid the distension was in large part due. The organs showed nothing unusual, except that the urinary bladder was

<sup>1</sup> Ohlmacher gives reference as follows: Auerbach, Ueber einen sexuellen Gegensatz in der chromophile der Keimsubstanzen; Sitzgsber. k. preuss. Akad. d. Wissensch. Berlin, June 25, 1891, pp. 713-750; Adamkiewicz, Untersuchung ii. d. Krebs u. d. Princip. seiner Behandlung, Wien u. Leipzig, 1893; Noeggerath, Beiträge z. Struktur u. Entwicklung d. Carcinoms, Wiesbaden, 1892; Watasé, Journ. Morphol., 1892. vi, pp. 481-493.

largely distended and the kidneys were twice the normal size. Ovaries moderately developed, but not sufficiently to account for the abnormal distension. Besides the *Myxosporidia*, the kidneys showed an extensive invasion of bacteria.

*Effects.*—There can, Ohlmacher says, be scarcely any doubt that the *Myxosporidia* were the direct factors in the pathologic changes. Their number was very great, the tubules of both kidneys being filled. The mere mechanical effect must have been obstruction of secretion and as a remote result ascites and general oedema. Undoubtedly the presence of large numbers of bacteria (to be regarded as a secondary infection) was a potent factor in hastening death.

Subsequent comparisons with sections of the kidneys of other toads show the tubules in the first toad to have been dilated and their lining cells to have been flattened and less rich in protoplasmic material than normal. The kidneys of the 3 comparatively slightly infected toads collected in July, 1893, showed no macroscopic lesions. Microscopically no bacteria could be found. The absence of the bacteria, Dr. Ohlmacher thinks, probably had as much weight in determining the comparative innocuity as the smallness of the number of *Myxosporidia* (letter, 1893).

Through the kindness of Dr. Ohlmacher I have been enabled to examine his specimens, and can add the following:

*Orientation of the spore.*—The capsules are 2, in 1 group, anterior; valve-junction plane, vertical; shorter axis of spore, antero-posterior; longer axis, transverse. Sporoplasm showing no evidence of a vacuole, even in iodine-stained sections. Beyond a slight median notch in its posterior border (produced, I believe, by a slight inward, as well as outward, projection of the ridge), I was not able to find any evidence of sporoplasm-segmentation, and am therefore compelled to regard this as an optical illusion, produced by the overlying ridge and reinforced by the posterior median notch.

This orientation necessitates the reference of this species to *Chloromyxum* (*Sphaerospora*). From *C. (S.) elegans* it is distinguished by its transversely elliptic outline and its dimensions. The fact of its identical organal distribution (renal tubules) should also be noted.

Finally, Dr. J. B. Whinery has recently published the results of a careful detailed restudy of this species. He gives the following table, showing the equivalence of Ohlmacher's nomenclature with that I have adopted:

Ohlmacher's term.	Present equivalent.
Capsule.....	Shell.
Pole corpuscle.....	Capsule.
Plasmatic mass.....	Sporoplasm.
Projectile thread.....	Filament.
Sides.....	Anterior and posterior ends.
Ends.....	Sides.
Vacuole.....	Pericystic space.

From Dr. Whinery's paper the following data are condensed:

[Page 660] All the toads examined (about a dozen in all) were from Sycamore, De Kalb County, 60 miles west of Chicago. The toads were kept in the laboratory sink, and taken from this, from time to time, for examination.

The extent of the infection must vary with the surroundings and environment of the animals. Seven toads examined—2 males and 5 females—showed 1 male and 4 females infected. It is quite probable that the mortality was increased by the confinement in a comparatively small space. During the confinement the toads became stupid, moved about but little, and in 2 or 3 days began to die, 1 dying every day or two. Some of them lived about 3 weeks. Before death no change in external appearance was noticed, except in some cases a distension of the abdomen. Post mortem some increase in amount of peritoneal fluid was usually noticed, but in the toads examined by Whinery this was never so large in amount as in the toad examined by Ohlmacher. The abdominal viscera showed signs of congestion; the intestines being usually distended with gas and the kidneys enlarged and in a congested state. The parasites were found only in the tubules and in the urinary bladder, and in the spore stage. Ohlmacher's view that they probably kill by mechanical pressure seems very plausible on account of the large number of parasites in the tubules.

[Page 661] This number varies in different specimens; sometimes only scattering tubules, in other cases large areas of tubules being filled with parasites. They were never found in the glomeruli or epithelial cells. In the bladder they were found in the folds of the mucous membrane. Ohlmacher has found them in urine collected during chloroform narcosis, in a clean basin.

*Detailed Morphology of Spore.*—Length about 6  $\mu$ ; breadth about 8  $\mu$ ; size slightly varying in the same preparation. Shape, slightly oval. Shell, showing a distinct striation, the stria appearing to proceed from the shell of each lateral half and to center at the valve-junction, midway between the anterior and posterior ends. Spore showing at each end a slight projection,<sup>1</sup> running between which 2 points is the faint transparent ridge, marking the valve-junction. The projections represent the vertical optical section of the ridge. The spore is thus composed of 2 valves, their junction plane dividing the spore into 2 symmetrical halves. Two small knob-like thickenings (which show well in the fresh, unstained spore) can be seen at the anterior projection, 1 belonging to each valve. The spores often show cleavage at the anterior end along the line of the valve-junction. Capsules 2, round, 3  $\mu$  to 3.5  $\mu$  on an average, situate at the anterior end, 1 in each valve. A filament arises from each capsule, and, penetrating the shell, leaves the spore at the anterior end. The capsules seem to have the power of projecting and drawing in these filaments. Length of filaments often more than 4 to 8 times the diameter of the spore. Just after entering the spore, before reaching the capsule, they often appear in a spiral roll preparatory to being coiled in the capsule. Sporoplasm situated in the posterior end, extending to the sides, in form approaching a crescent; not completely filling the space posterior to the capsules; under high powers ( $\frac{1}{2}$  Leitz) appearing homogeneous and finely granular; showing in fresh preparations the more highly refractive granules designated nuclei by Thélohan; these apparently vary in number and position in fresh spores, and never appear in hardened and stained preparations.<sup>2</sup> A vacuole could not be discovered in this species.

<sup>1</sup> "Termed by Gurley the 'mucronate [mucronate] projection.'" This name was employed by me in a letter in a general sense only (a mucronate projection) and was not intended as an additional special term.

<sup>2</sup> Ohlmacher had only hardened material, a fact which, Whinery thinks, explains his failure to find nuclei. I can not believe, from Dr. Whinery's description, that the bodies he calls "nuclei" are really such, since they *disappear entirely* in hardened and stained specimens. Although I have not seen Dr. Whinery's material, I venture to suggest the possibility of their being fat globules.

*Micro-chemistry.*—The parasites were studied fresh (by teasing kidney tissue, and examining this in a hanging drop, or in fluid media of different kinds), and also after treatment with various fixing and staining agents. In the fresh state, a dilute solution of potassium hydrate caused a swelling of the spore, and brought out the shell and filaments plainly. Glycerin acts well as a medium for the examination of the fresh spore. Probably the best medium to use for the hanging drop is toad's urine. Iodine (aqueous solution) colors the spore a uniform brown. In fixing cover-glass preparations, no advantage was gained by fixing them in alcohol and ether, or in osmic acid, over that obtained by passing the covers through a flame. In the fresh state the filaments were made plainer in fixed cover-glass preparations [Page 662.] by a number of reagents. Aqueous methyl blue and Babes' anilin water safranin bring the filaments into view quite satisfactorily. As fixing agents, Flemming's solution, Heidenhain's mercuric chloride solution, absolute alcohol, Carnoy's acetic alcohol, and Perenyi's fluid were tried, the first and last being found unsuitable on account of the production of shrinkage and distortion. The fixed material was imbedded in xylol paraffin by the usual methods. Numerous separate and combined stains were employed with varying results, the capsules with almost all stains showing the greatest affinity for the coloring matter, the degree of affinity varying somewhat in different spores. Pfitzner's safranin is especially good, with a striking affinity for the capsules. Ohlmacher's dichromophilism was demonstrated with fuchsin and iodine green (Russell's method), and with safranin and methyl blue (Ohlmacher's method). "This chromophilous reaction is a very striking and possibly significant phenomenon in these organisms."

**90. *Chloromyxum* (*Sphærospora*) *perlatus* Gurley, 1893. Pl. 40, fig. 2.**

(Psorosperm of *Acerina cernua*, Balbiani, 1883, Journ. de Microgr., VII, pp. 201, 204, fig. 44; *ib.*, Balbiani, 1884, Leçons sur les Sporozoaires, p. 133, fig. 40.)

*Myxobolus perlatus*, Bull. U. S. Fish Com. for 1891, XI, p. 415; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

No description (see also p. 265).

*Habitat.*—On *Acerina cernua* L.

**91. *Chloromyxum* (*Sphærospora*?) *sp. incert.* Pl. 40, fig. 3.**

Spore of *Lota vulgaris*, Bütschli, 1882, Bronn's Thier-Reich., I, pl. 38, fig. 22.

Cyst unknown.

*Myxosporidium.*—Not described. The sporoblast produces a single spore?<sup>1</sup>

*Spore.*—Not described. For the reasons given on p. 265, the present generic reference of this species is probably the correct one, and the species should be closely compared with *C. mucronatum*.

*Habitat.*—Ovary of *Lota lota* L. (= *vulgaris*); ling.

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<sup>1</sup> "Each spore in a special transparent membrane."



92. *Chloromyxum* (*Sphærospora*) *dujardini* Thélohan, 1892. Pl. 40, figs. 4-7.

Cyprinus rutilus, "psorospermus" of.	Cyprinus erythrophthalmus, "psorospermus" of.	dujardini.	Date.	Authority; reference.
×	×	-----	1841	Müller, Müller's Arch., pp. 481, 486, pl. 16, fig. 4b, c.
×	×	-----	1843	Müller, Rayer's Archiv. Méd. Comp., I, p. 226, pl. 9, fig. 4b, c.
×		-----	1843	Rayer, Rayer's Archiv. Méd. Comp., I, p. 269.
(pars.)	×	-----	1845	Dujardin, Hist. Nat. des Helminthes, p. 644, pl. 12, fig. 12 N <sub>1</sub> , 12 N <sub>2</sub> .
×	×	-----	1853	Robin, Hist. Nat. Végét. Par., p. 299, pl. 14, fig. 6.
(pars.)		-----	1882	Bütschli, Bronn's Thier-Reich., I, pl. 38, fig. 5.
×		-----	1892	Thélohan, Bull. Soc. philomat. Paris, IV, p. 175.
		Myxosoma et Mixosoma ..	1893	Gurley, Bull. U. S. Fish. Com., XI, p. 419.
		Chloromyxum	1893	Braun, Centralbl. Bakt. u. Parasitenkde, XIV, p. 739.
		Myxosoma ..	1893	Braun, Centralbl. Bakt. u. Parasitenkde, XIV, p. 739.
		Chloromyxum	1894	Braun, Centralbl. Bakt. u. Parasitenkde, XV, p. 87.

*Synonymy*.—The first 6 references in the table, except those to Dujardin and to Bütschli, represent the same form, the later being mere copies of Müller. The fusion of the form observed by Dujardin with that observed by Müller is on the authority of Thélohan, who states (letter to the author, 1893) that he has observed his *Myxosoma dujardini* upon both *Leuciscus rutilus* and *L. erythrophthalmus*, and that he believes that Müller's and Dujardin's figures represent the same species. Bütschli's form is also probably referable here; size of the last, 0.46 mm.

Concerning the form observed by him in *Leuciscus rutilus*, Müller says:

Once there was found on the pseudobranchias (*Nebenkiemen*) a mass of small yellow cysts. The size of this mass was 4 lines. This time all the cysts contained elongate capsules [spores] with pointed anterior and bluntly rounded posterior ends (fig. 4b). On the flat border the convex surfaces were exactly equal and the 2 diverging vesicles were attached interiorly at their points.

Thus this form was never found coexisting in the same cyst with *Myxobolus cycloides*. Considering the great frequency of occurrence of the latter species such coexistence would be expected if they were merely different forms of one species. Their persistent nonassociation thus strongly reinforces the argument in favor of their specific distinctness drawn from their different characters.

Cyst not described.

*Myxosporidium*.—Spores imbedded in and held together by an almost diaphanous, ramified, glutinous mass, 1.25 to 1.50 mm. long, decomposable by water, analogous to the amœbæ, apparently destitute of an envelope (Dujardin).

*Spore*.—Oval, pointed anteriorly, broadly rounded posteriorly, length, 10 to 12  $\mu$  (0.0051''' to 0.0054'''); breadth, 7  $\mu$  (0.0034''') untailed; capsules 2, of equal size (Müller).

*Habitat*.—Encysted in the pseudobranchiæ of *Leuciscus rutilus* from German rivers; branchial lamellæ of *Leuciscus* (*Scardinius*) *erythrophthalmus* from the Vilaine, at Rennes, France.

## V. CERATOMYXA Thélohan, 1892.

Etymology not given.

Bull. Soc. philomat. Paris, IV, pp. 169, 171, 175; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, pp. 411-12, 420; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 738-9; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

*Definition.*—*Chloromyxidæ* with bilaterally symmetrical, transversely extended, subisosceles-triangular spores whose breadth greatly exceeds the length; valves hollow-conical with solid tips; sporoplasm unilaterally and asymmetrically situated; type, *C. sphaerulosa*.

The position of this genus in the system depends upon the interpretation of its symmetry. Admitting (as we may safely do) that the position of the capsules marks the anterior extremity, the question arises whether the plane of junction of the valves is the vertical or the longitudinal. If it be vertical, we then have: (1) Vertical plane intercapsular; (2) spore laterally extended; (3) valves bilaterally subsymmetrical; (4) decided sporoplasmic bilateral asymmetry.

On the other hand the supposition that this plane corresponds to the longitudinal necessitates the following suppositions: (1) That the vertical plane can be *percapsular*; (2) that the spore is vertically extended; (3) valves superior and inferiorly subsymmetrical; (4) decided (sporoplasmic) supero-inferior asymmetry.

While admitting the striking anomaly exhibited by this species in its bilaterally asymmetric distribution of the sporoplasm (which certainly warrants its generic separation), it seems more easy to accept this than to admit (*a*) that the longitudinal plane can be *percapsular*,<sup>1</sup> and (*b*) that the spore is greatly extended supero-inferiorly, of neither of which conditions any other known species exhibits an example. There are, however, species which exhibit, though in a less degree, bilateral asymmetry (*Myxobolus unicapsulatus*, *M. inequalis*, *M. strongylurus*).

Two other characters should be noted. As in the other forms habitant in the fluid-filled organs, the *Ceratomyxa* species are never seen "encysted." Further, 3 out of the 4 known species possess the striking peculiarity of *bisporogenesis*, each myxosporidium producing only 2 spores. The fourth species presumably (from Thélohan's silence) does not possess this character. It is well to note that this character is possessed by only one other species, viz: Perugia's *Myxosporidium merlucii*, a gall-bladder species provisionally and doubtfully referred to *Myxobolus* (see p. 242).

Finally, while this paper was passing through the press, M. Thélohan's recent paper<sup>2</sup> was seen. It seems to imply very strongly two things,

<sup>1</sup> No known instance exists of 2 capsules being placed one above the other (i. e., in the vertical plane, which would thus be percapsular). The only species in which by any possibility the vertical plane could be asserted to be percapsular is *Cystodiscus? diploxyis*, but here the condition is at least equally well (and I think much better) explained on the view that the *intercapsular* plane is the vertical.

<sup>2</sup> Compt. Rend. Acad. Sci. Paris, 1894, CXVIII, pp. 428-430.

viz: (1) That bisporogenesis must be admitted as a (very striking) generic feature; and (2) that if, as Perugia asserts, *Myxobolus merluccii* possesses this character, it is in all probability a *Ceratomyxa*, and not a *Myxobolus*. And two facts confirm this latter view, viz: The improbability in *Myxobolus* of a gall-bladder habitat and the rarity of spores whose breadth exceeds the length. Perugia's species is, however, provisionally left under *Myxobolus*, on account of his positive statement as to the presence of an iodophile vacuole.

The following is an abstract of Thélohan's paper:

[Page 429] Besides the species formerly published<sup>1</sup> in which the myxosporidium produces but 2 spores, I have since confirmed the same peculiarity in a rather large number of new forms in the gall-bladders of certain Mediterranean fishes. All these 2-sporing species belong to my family "Myxidiées," the greater part of them being clearly referable to *Ceratomyxa*, while the others, by successive modifications of spore-form, establish a transition between that genus and *Sphaerospora*. This last connects the 2-sporing species with the many-sporing, and at the same time, by its habitat, the free species to the tissue-imbedded forms.

There is thus no absolute separation between the 2-sporing and the other *Myxosporidia*. The 2-sporing always live a free amœboid life in the bile-fluid and exhibit a very great motility, owing to specialized pseudopodia heretofore described.

These 2-sporing *Myxosporidia* with localized pseudopodia and rapid movements represent the most elevated type of organization. As regards the interpretation of the facts, are they perfected types derived from inferior, or are they [Page 430] the primitive type, the others, especially the tissue-imbedded species, being forms degraded by a more pronounced (a, so to speak, more intimate) parasitism? Thélohan favors the latter view. Great stress is to be laid upon the progressive increase in the number of spores occurring *pari passu* with degradation of form and increase of parasitism, such increase of reproductive elements being always one of the most constant attributes of parasitism.

**84. *Ceratomyxa arcuata* Thélohan, 1892.**

Compt. Rend. Acad. Sci. Paris, cxv, p. 1091.

Cyst none.

*Myxosporidium*.—Of variable form, diameter apparently not exceeding 35 or 40  $\mu$ ; destitute of prolongations. Endoplasm finely granular and homogeneous, containing some scattered fatty globules; destitute of spherules. Pseudopodia ectoplasmic, lobed; the filiform variety absent.

*Spore*.—Relatively very small; length, 5  $\mu$ ; breadth, 40  $\mu$ .

*Habitat*.—Gall-bladder of *Onus tricirratus* (= *Motella tricirrata*) collected at Roscoff, in August, 1892.

*Remarks*.—This differs from the other species of the genus principally in its much smaller size.

**85. *Ceratomyxa agilis* Thélohan, 1892.**

Compt. Rend. Acad. Sci. Paris, cxv, pp. 962-3.

*Myxosporidium*.—Attaining a maximum length of 85  $\mu$ , and a maximum breadth of 20  $\mu$ ; assuming various forms, most frequently elongated, subcylindric, a little swollen at the middle. One end (which on account of being constantly foremost in progression is to be regarded

<sup>1</sup> Compt. Rend. Acad. Sci. Paris, 1894, cxviii, pp. 428-430.

as the anterior), rounded; the other (posterior) usually attenuated, pointed, sometimes, however, swollen, rounded or bifurcate, or 7-, or 8- (or more) lobed. Limit between ectoplasm and endoplasm almost indistinguishable; myxoplasm finely granular, presenting constantly, near the anterior end, grouped in variable number, some small, very refringent, fatty globules.

Pseudopodia differing markedly from those of other *Myxosporidia*, always limited to anterior end; number variable up to 7 or 8, perfectly distinct from one another, almost filiform, progressively attenuating to their drawn-out pointed extremities; length very considerable, *ad max.* one half that of the myxosporidium; composed of exceedingly fine granular plasma resembling the ectoplasm of other *Myxosporidia*, whence their ectoplasmic nature may be inferred.

Movements of pseudopodia very rapid, describing a semicircle, always from before backward. Thélohan could not determine whether, upon arriving at their limit of backward motion, the pseudopodia fuse with the myxosporidium or move forward to repeat their sweep. Locomotion of myxosporidium thus produced, relatively rapid (3 times its length in 25 seconds). Remainder of myxosporidium motionless, apparently, however, possessing a certain contractility, as is seen when the anterior (pseudopodial) end becomes lodged against an obstacle.

*Spore*.—Similar to that of *Ceratomyxa sphaerulosa*; breadth 60  $\mu$ . Never more than 2 spores in one myxosporidium.

*Habitat*.—Free in the gall-bladder of *Dasyatis pastinica* L. (= *Trygon vulgaris*) sting-ray at Concarneau in September, 1892.

**86. *Ceratomyxa appendiculata* Thélohan, 1892.**

Compt. Rend. Acad. Sci. Paris, cxv, pp. 963-964.

Cyst none.

*Myxosporidium*.—Presenting special characters which clearly distinguish this species. Fully developed forms assume very irregular and very variable shapes; remarkable for the presence of 1 to 4 or 5 immovable prolongations, composed of an endoplasmic axis and an ectoplasmic covering, which extend out from a central portion of a very variable form. Length of prolongations may reach twice the diameter of the central portion. Pseudopodia lobed, originating from the ectoplasm of the central mass at no fixed point, which is changeable from moment to moment.

*Spore-formation*.—Taking place in the above-mentioned central portion, each myxosporidium producing 2 spores.

*Spore*.—Length (?), 5 to 8  $\mu$ ; breadth (?), 65  $\mu$ .

*Habitat*.—Free in the gall-bladder of *Lophius piscatorius* (angler) collected at Roscoff and at Le Croisic in August and September, 1892.

**87. *Ceratomyxa sphærulosa* Thélohan, 1892. Pl. 41, fig. 4.**

Bull. Soc. philomat. Paris, iv, pp. 171-3, 175, fig. 1; *ib.* Thélohan, 1892, Compt. Rend. Acad. Sci. Paris, cxv, pp. 961-2; *ib.* Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, p. 420; *ib.* Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xiv, pp. 738-9; *ib.* Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

Cyst, none.

*Myxosporidium*.—Spherical or ovoid; youngest stages exhibiting very distinct amœboid movements, colorless; older individuals yellowish, presenting a very remarkable constitution. Ectoplasm thin, emitting lobed pseudopodia, with very slow movements. Endoplasm appearing riddled with small (3 or 4  $\mu$ ) clear spheres between which lies a grayish, finely granular plasma. Spheres often exhibiting, grouped at their center, a variable number (most frequently 5 or 6) of small yellow, brown, or greenish granules which resist nitric acid and potassium hydrate longer than the spheres which envelop them. Thélohan was unable to express any opinion as to the nature of the spheres, which, he remarks, constitute one of the most remarkable peculiarities of this species.

*Spore formation*.—Each myxosporidium forms at the most 2 spores; never more. Solid distal portion of valve folded back along the posterior border during development. Thélohan notes the similarity in this respect to the development in the tailed *Myxobolus* species (see p. 248) and says that the anterior convexity of the curve presented by the long (transverse) axis seems the effect of this primitive arrangement.

*Spore*.—Transversely extended, symmetrically (or subsymmetrically) double scalene-triangular; length, 8 to 10 or 12  $\mu$ ; breadth, 90 to 100  $\mu$ .<sup>1</sup> Shell bivalve; valves right and left; symmetrical or subsymmetrical; shape of each valve hollow-conical, with the distal extremity solid for a variable distance; valves united along the cone bases, a slender ridge marking their line of junction. The shell cavity thus consisting of 2 (lateral) halves, one of which is always occupied by a variable number of small very pale masses whose exact nature is unknown, but which seem to represent the residue of capsule formation.

*Sporoplasm*.—Constantly situated in the other half of the shell cavity, of which it occupies only a relatively very small portion; finely granular; no iodophile vacuole.

*Capsules*.—Two, the largest known, filament very clearly seen, coiled; extrusion easily produced by potassium hydrate or ether, each capsule presenting as a rule a special opening placed on one side of the suture.

*Habitat*.—Gall bladder (free floating in bile) of *Galeus mustelus* (= *Mustelus vulgaris*) smooth dogfish and of *Galeorhinus galeus* (= *Galeus canis*) taken at Valéry-au Caux, by Balbiani, in August, 1891.

<sup>1</sup>Thélohan gives the dimensions reversed (*i. e.*, as length 100, breadth 8 to 10 or 12  $\mu$ ) but this is of course a wrong orientation. Similarly with other species.

Fam. CYSTODISCIDÆ Gurley, 1893.

Bull. U. S. Fish Com. for 1891, XI, pp. 412-13; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Definition.*—*Phanocystes* whose spores possess antero-posterior and bilateral symmetry; capsules in 2 groups situated at the (anterior and posterior) ends; a bivalve shell, the plane of junction of whose valves is perpendicular to the longitudinal plane; condition of sporoplasm unknown; type genus *Cystodiscus*.

To the family as thus defined, I have provisionally (by way of taxonomic necessity) approximated Thélohan's genus *Spharomyxa*. It is characterized, Thélohan says, by the structure of the spores, especially by the form of the filaments and their disposition in the capsule. In the absence of figures, the orientation of the spore, upon which classification must be based, is uncertain. The double grouping of the capsules necessitates the approximation (at least among known genera) of this genus to *Myxidium* or to *Cystodiscus*. Between the last two, the presence of a membrane around the myxosporidium and especially the bivalve structure of the spore would seem (at a taxonomic guess) rather to approximate *Spharomyxa* to *Cystodiscus*.

It may be frankly admitted that, as at present composed, this family is somewhat unsatisfactory and must be held subject to revision, probably in the direction of elision. For of the species with the capsules in 2 groups we now know (excluding *Myxidium* ? sp. 102, about which hardly any data exist) 5 species: *Cystodiscus immersus*, *Cystodiscus* ?? *diploxyis*, *Spharomyxa balbianii*, *Myxidium lieberkühnii*, *Myxidium* ? *incurvatum*. Of these *M. lieberkühnii* presents a sufficiently distinct group of characters to warrant its delimitation as the type of a family. The other 4 species then agree in two very important characters, viz:

1. Arrangement of capsules in 2 groups.
2. Presence of a bivalve shell.

Further than this, however, our analysis can not, for want of data, be at present safely pushed. Indeed, I have even left *Myxidium* ? *incurvatum* under *Myxidium* (where in all probability it does not belong) rather than place it elsewhere at random. Obviously the next step is the determination of the 3 symmetry planes and the orientation of the valve-junction plane. I suspect the future will separate generically *C.* ?? *diploxyis* from *C. immersus*, the former appearing to have the valve-junction plane parallel and the latter to have it perpendicular to the longitudinal plane. In the present uncertainty, however, especially as long as the symmetry-relations of *Spharomyxa* are so dubious, the present provisional arrangement is probably preferable to another new genus, and perhaps a family.

## VII. CYSTODISCUS Lutz, 1889.

Etymology not given.

Centralbl. f. Bakt. u. Parasitenkde, v, p. 88; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, pp. 411-13; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

*Definition.*—Characters those of the family; type, *C. immersus*.

Whatever may be the ultimate taxonomic destination of the species here included, the genus will, I think, stand, as it is the first in order of priority, having the spore with the capsules in 2 groups, and a bivalve shell.

**97. *Cystodiscus immersus* Lutz, 1889. Pl. 42, figs. 1-10.**

Centralbl. f. Bakt. u. Parasitenkde, v, pp. 84-88, figs. 1-10 separately and subsequently; *ib.*, Gurley, 1893, Bull. U. S. Fish Com. for 1891, xi, p. 413; *ib.*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 87.

Cyst none.

*Myxosporidium.*—Youngest forms unknown. Hoping to find them in the tadpoles, Lutz examined about a dozen, but the gall-bladders were entirely free; in frogs and toads only a little larger, however, myxosporidia were found, but they (even the very small ones, less than 0.1 mm. in diameter) already showed the stiff disk form. In number, usually several, often very many (30 to 50), visible through the bladder wall, appearing macroscopically as round transparent disks or leaflets, as thin as paper, with frequently a whitish border in which the upper and under surfaces meet directly (without the intervention of a lateral surface as in a cylinder); upper and under surfaces very slightly convex, the thickness being only  $\frac{1}{20}$  to  $\frac{1}{10}$  of the diameter; body-form thus feebly biconvex lenticular, ranging in diameter from the limits of visibility to 1.5 or 2 mm.

Ectoplasm forming a plainly perceptible, transparent, structureless membrane, completely resistant to the bile and noticeably so to chemical reagents, disintegrating on prolonged immersion in water; preserving the form of the organism which otherwise almost certainly would, on account of its great thinness, become wrinkled and folded, but whose borders have a subcircular outline. Ectoplasm often containing great numbers of micrococcus-like bodies, which, as they brown only very slightly with osmic acid, can scarcely be pure fat. They also can not be cell-nuclei.

Endoplasm containing numerous large vesicles, polygonal-flattened by mutual pressure, producing the appearance of a cellular structure. Vesicles possessing a subglobular contour, showing no trace of a nucleus; upon rupture of the ectoplasm, escaping spontaneously into the bile, in which (also in alkaline solutions) they immediately vanish under the eyes of the observer, probably on account of the solution of a delicate surrounding membrane and the subsequent solution of their contents. Amœboid movements are completely excluded by the mem-

branous character of the ectoplasm. No traces of change of form or place were seen.

*Spore formation.*—Beginning with individuals scarcely one-tenth the maximum size, the number of spores being then, however, relatively as well as absolutely less; number increasing *pari passu* with growth, individuals of equal size not necessarily showing, however, equal numbers. In specimens largest and most rich in spores the latter show themselves scattered over the surface at very short intervals, while on the borders they form a compact zone visible macroscopically as a white ring.

Pansporoblast?: Myxosporidia of various ages tolerably frequently show a spore-foundation [*Sporenanlage*] in the form of a smaller, more elongate, and only delicately outlined oval, containing two small pale perfectly round capsules (somewhat removed from the poles), which inclose a tolerably large dark biconcave-ended cylindrical rest-body (*Restkörper*). The delicately outlined oval contracts its bulk, its outline clears up, and the shell and capsules become thicker and very prominent. Valve-connection takes place through a process of the shell, and the spore becomes more ventricose.

*Spore.*—Lying outside the vesicles, always arranged in pairs, the latter rather irregularly scattered under and only loosely connected with the ectoplasm, concentrated in greatest numbers along the borders, forming a white ring. Length of mature spore, 12 to 14  $\mu$ ; breadth, 9 to 10  $\mu$ ; regularly oval, with blunt ends; spore showing no independent movements except filament extrusion.

Shell rather thick and firm, indistinctly and finely transversely striate, possessing the usual resistance to chemical reagents; bivalve, the valve-junction plane oblique (like the diagonal of a rectangle), inclined about 45° to the "equatorial" [transverse?] plane. This condition doubtless stands, Lutz says, in connection with the position of the capsules at either end, one valve lodging each. Around the border of each valve is placed, hoop-like, a little elastic rod, plainly projecting in profile, rebounding, when treated with potassium hydrate, in the form of a more or less extended band, the valves thereby becoming loosened, a piece often being torn away. Lutz remarks that these observations agree with Balbiani's (p. 223). Lutz, however, never saw any connection of spore-pairs through the medium of the loosened bands.

Capsules 2, separated, 1 at each end, subglobular-pyriform, slightly sharper anteriorly, glittering strongly in water or in bile, only slightly so in glycerin and other refractile fluids; size diminished by extrusion of filaments, walls plainly double-contoured. Filaments difficultly perceivable when fully coiled, plainly visible when half uncoiled; extrusion frequent in bile, not so common in water; extrusion also producible by various reagents, most certainly by potassium hydrate. Length, 4 to 5 times that of the spore-length.

Sporoplasm transparent, first becoming plainly visible after the action



of coagulants, as an irregular, very low and biconcave-excavated cylinder. Lutz could find no true nuclei, either before or after development. Micrococcus-like corpuscles (similar to those in the ectoplasm, see above) were present, but on account of their inconstancy, these must be regarded as plasmatic secretions.

*Exit of sporoplasm.*—Never observed, prolonged immersion in water producing only a gaping of the valves, with or without a falling out of the capsules.

*Habitat, etc.*—Gall-bladder (free-floating in and escaping with the bile) of *Bufo aqua* (toad) in every one of 50 half grown to grown individuals taken at the most various times at one locality in Brazil: parasites mostly multiple, sometimes as many as 50; also in young specimens of *Cystignathus ocellatus* (toad) from 2 localities in Brazil. On the contrary they were absent from 2 large individuals of *Bufo aqua* from other provinces of Brazil. They were also absent from all the tadpoles examined and from metamorphosed toads from several localities.

*Effects.*—The myxosporidia observed appeared in nowise to impair the histological integrity of the gall-bladder.

98. *Cystodiscus* ? ? *diploxys* Gurley, 1893. Pl. 42, figs. 11–13.

Pyraxis (or Tortrix) viridana, psorosperms of.	diploxys.	Date.	Authority ; reference.
×	-----	1866	Balbiani, Journ. Anat. et Physiol., Paris, III, pp. 600–2.
×	-----	1867	Balbani, Journ. Anat. et Physiol., Paris, IV, pp. 275, 276, 335 (footnote), pl. 12, figs. 10–12.
×	-----	1882	Bütschli, Bronn's Thier-Reich, I, p. 590.
×	-----	1890	Pfeiffer, Virchow's Arch. f. path. Anat. u. Physiol., CXXII, p. 559.
×	-----	1890	Thélohan, Annal. d. Microgr., Paris, II, p. 193.
×	-----	1892	Henneguy and Thélohan, Compt. Rend. hebdom. Soc. Biol. Paris, IV, p. 587.
×	-----	1893	Perrier, Traité de Zool., p. 459.
-----	Cystodiscus ?	1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, pp. 411–13.
×	-----	1893	Braun, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739.
-----	Cystodiscus ?	1894	Braun, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

*Cyst.*—Spherical, 12 to 15 (in 1 individual 4) in number, 230 to 400  $\mu$ . Membrane rather thick. Contents rounded masses composed of fine brownish granulations suspended in a viscid homogeneous liquid. In 1 cyst (pl. 42, fig. 12) the parasites were mixed with numerous fat-like globules, insoluble in caustic soda; coloring wine red with iodine.

*Spore.*—Greatly resembling the “psorosperms” of fishes; elliptic or slightly flattened, traversed by a ridge apparently marking the line of valve junction. Sometimes showing 2 small brilliant twin grains placed at one of their extremities, sometimes 4 grains disposed in pairs at the 2 “ends”; not visibly affected by concentrated alkalis or feeble acids; becoming brilliant and homogeneous in salt water.

*Habitat.*—In the free state or inclosed in great spherical cysts in the abdominal cavity of the butterfly of *Tortrix viridana* (an insect).

Concerning this species Bütschli says:

Balbani has observed cysts in the body cavity of a butterfly (*Pyrallis viridiana*) which were filled with corpuscles possessing a structure similar to that of the myxosporidian spore. The observation is, however, not sufficient to demonstrate that it belongs to the *Myxosporidia*.

Thélohan and Henneguy regard it as a myxosporidian, and it is difficult for me to think otherwise.

#### VIII. SPHÆROMYXA Thélohan, 1892.

Etymology not given.

Compt. Rend. Acad. Sci. Paris, cxv, p. 1093; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 737.

*Definition*.—Characters to be inferred from those of the type species, *S. balbianii*.

After several vain attempts to draw up a satisfactory generic definition as between this genus and *Cystodiscus*, I have concluded that at present there are not in the record sufficient data for their accurate delimitation.

##### 99. *Sphæromyxa balbianii* Thélohan, 1892.

Compt. Rend. Acad. Sci. Paris, cxv, pp. 1091-3; *ib.*, Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, xv, p. 738.

*Myxosporidium*.—Generally visible to the naked eye as a small opaque, more or less regular, usually subspherical mass, occupying a variable part of the bladder and escaping with the bile; yellowish or greenish-yellow, of a relatively firm consistence, permitting of handling. Attempts at teasing render evident the presence of a thin membrane. Under the microscope the myxosporidium shows absolutely exceptional characters. Ectoplasm forming a clear, homogeneous zone, presenting in sections a very clear striation. Endoplasm more granular, inclosing numerous spores.

*Spore*.—Resembling that of *Myxidium lieberkühni*, elongate, slightly swollen at middle; extremities abruptly truncate, cut squarely off, so to speak, so as to present very sharp "lateral" angles; "length" [?] 13 to 16  $\mu$ ; "breadth" [?] 5  $\mu$ . Shell bivalve, finely striate, parallel to the longer axis. Capsules 2, one at each "extremity," their axes oblique and oppositely directed with reference to the longer [transverse?] diameter of the spore. Filament very peculiar, forming a relatively very short (average length 15  $\mu$ ) cone, the diameter of whose base nearly equals the breadth of the extremity of the spore. Exit produced by iodine water, potassium hydrate, sulphuric acid, etc. The mode of coiling is equally peculiar, the axis of the coil being perpendicular to the long axis of the capsule. Sporoplasm forming a single mass, destitute of an iodophile vacuole; nuclei, 2; the pericapsular nuclei (Thélohan's "nuclei of the capsulogenous cellule") are also present.

*Habitat*.—Free in the gall bladder of *Onus tricirratum* and *O. maculatus* (= *Motella tricirrata* and *M. maculata*); very common, especially at Roscoff.

Fam. MYXIDIIDÆ Gurley, 1893.

("Myxidiées" (*pars*) Thélohan, 1892, Bull. Soc. philomat. Paris, IV, pp. 173, 175); *Myxidiida*, Bull. U. S. Fish Com. for 1891, XI, pp. 412, 420; *Myxidiea* [Thél.] Braun, 1893, Centralbl. f. Bakt. u. Parasitenkde, XIV, p. 739; *Myxidiida*, Braun, 1894, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

**Definition** (provisional as regards negative characters).—*Phænocystes* destitute of antero-posterior, but possessing bilateral symmetry; capsules in 2 groups in the (right and left) wings; no bivalve shell; no vacuole; type (and only) genus *Myxidium*.

## IX. MYXIDIUM Bütschli, 1882.

Etymology not given.

Bronn's Thier-Reich, I, pl. 38; *ib.*, Lankester, 1885, Encycl. Britan., 9 ed., XIX, p. 855; *ib.*, Thélohan, 1892, Bull. Soc. philomat. Paris, IV, p. 175; *ib.*, Weltner, 1892, Sitzgsber. Ges. Naturf. Freunde Berlin, p. 351; *ib.*, Perrier, 1893, Traité de Zool., p. 460.

**Definition.**—Characters those of the family; type, *M. lieberkühnii*.

100. *Myxidium lieberkühnii* Bütschli, 1882. Pls. 43-46; pl. 47, figs. 1-5.

Esox lucius "psorosperms" etc., of.	lieberkühnii.	esocis.	Date.	Authority; reference.
×			1854	Lieberkühn, Müller's Archiv., pp. 5, 6, 349-52, pl. 14, figs. 1-4.
×			1854	Lieberkühn, Bull. Acad. Roy. Belg., XXI, pt. 2, p. 23.
×			1879	Leuckart, Parasiten des Menschen, p. 246, fig. 98.
×			1880	Gabriel, Jahres-Ber. schles. Gesellsch. f. vaterl. Cultur f. d. J. 1879, LVII, pp. 188-95.
×			1881	Bütschli, Ztschr. f. wiss. Zool., XXXV, pp. 638-48, pl. 31, figs. 25-40.
×			1882	Zoolog. Record for 1881, XVIII, Prot., pp. 34-35.
	Myxidium.		1882	Bütschli, Bronn's Thier-Reich, I, pp. 593-5, pl. 38, figs. 12-15.
×			1883	Balbani, Journ. de Microgr., VII, pp. 200-1, 274-5, fig. 64.
×			1884	Balbani, Leçons sur les Sporozoaires, pp. 126, 129-30, fig. 45.
	Myxidium.		1885	Lankester, Encyclop. Britan., 9 ed., XIX, p. 855, fig. xvii, 34.
×			1886	Leuckart, Parasites of Man, 2 ed., p. 196, fig. 98.
		Psorospermium.	1887	Koch, Encyklop. d. gesamt. Thierheilkde u. Thierzucht, IV, p. 94, fig. 668, 1.
×			1888	Pfeiffer, Zeitschr. f. Hygien. Leipzig, IV, p. 409.
×			1890	Thélohan, Annal. de Microgr. II, p. 198.
×			1890	Pfeiffer, Archiv. f. pathol. Anat. u. Physiol., CXXII, pp. 559-60.
×			1890	Pfeiffer, Die Protozoen als Krankheitserreger, 1 ed., pp. 41-9, 55, 98, figs. 12, 13, 15, table, figs. 1-11.
×			1891	Pfeiffer, Die Protozoen als Krankheitserreger, 2 ed., pp. 29, 91, 105, 127-33, figs. 52, 53, 55.
	Myxidium.		1892	Thélohan, Bull. Soc. philomat. Paris, IV, pp. 166, 169, 175.
	Myxidium.		1892	Engler & Prantl, Die natürlich. Pflanzenfamilien, Leipzig, Lfrg. 76, fig. 22.
×			1893	Perrier, Traité de Zool., p. 459-60.
×			1893	Oehlacher, Journ. Amer. Med. Assoc., XX, p. 562.
	Myxidium.		1893	Gurley, Bull. U. S. Fish Com. for 1891, XI, pp. 410, 420.
	Myxidium.		1893	Braun, Centralbl. f. Bakt. u. Parasitenkde, XIV, pp. 738-9.
	Myxidium.		1894	Braun, Centralbl. f. Bakt. u. Parasitenkde, XV, p. 87.

\* Of air bladder; error.

The description is based upon the (in the main) accordant results of Lieberkühn, Balbiani, Bütschli, and Pfeiffer, particularly upon those of the last two observers. Gabriel's accordant results have been incorporated, his divergent ones mostly footnoted.

*Life-history* (Pfeiffer).—Emerging from the spore, the young myxosporidium (until now the sporoplasm) next penetrates into the interior of the red blood corpuscles or of the cells of the bladder epithelium. Its intracellular existence continues until its increasing size ruptures the cell wall, when it escapes, differentiates its own protective ectoplasmic layer, and resumes amœboid movements. Finally endogenous (pansporoblastic) spore formation takes place, the spores ultimately become free, and the life-cycle is complete.

Cyst none.

*Myxosporidium*.<sup>1</sup>—Form varying much with age; at exit from spore globular-amœboid: while within, and at the time of exit from the epithelial and red blood cells, roundish; older forms cylindrical, ribbon or club shaped, or irregularly amœboid, presenting a very grotesque appearance, with branches, forkings, and long appendages. Size varying with age up to a maximum length of 300  $\mu$  (Bütschli) by a breadth of 136  $\mu$ . Youngest myxosporidia colorless; older ones colored yellowish or reddish or brownish-red by inclusions of extraneous pigment in the endoplasm. Myxoplasm, in all but the youngest stages, presenting a clear differentiation of ectoplasm and endoplasm.

Ectoplasm forming a rather thick, very transparent, colorless, delicate, finely granular layer, containing none of the characteristic endoplasmic elements; end in contact with the mucous membrane, colorless, destitute of granules, leafy or pronged for attachment. Opposite end richest in granules and in pigment, free-floating, usually rounded; free-floating forms partly agreeing with the above, differing, however, in being destitute of pronged processes, showing at times some peculiar differentiations, particularly the appearance shown on pl. 44, fig. 3, where it seems permeated by a system of canals. One end of body often more or less plainly radiate-striate, the usual distinction between the ectoplasm and endoplasm being here absent. This Prof. Bütschli regards as the attached (pronged) end. Also not rarely are seen a series

<sup>1</sup> Gabriel believed that the bladder does not furnish a suitable environment for metasporal development, consequently the latter must, he thinks, take place in or *via* the external world. In his opinion the myxosporidia living within the bladder represents not normally developing, but progressively degenerating forms. Such development as occurs within the bladder, by which apparently the way has been prepared for the replacement, at least within certain limits, of the perishing mother organisms, does not exclude the possibility of ripe spore-containers or free spores finding their way to the outer world and there under favorable (but as yet unknown) conditions developing. This supposition, a necessary postulate, becomes a certainty when it is remembered that only thus [by active or passive migration] could the parasite have reached the bladder. Probably repeated, though perhaps (as indicated by the variations in their occurrence) not continuous, infection-immigrations occur.

of dark, longitudinal, ectoplasmic laminae separated by clear, somewhat reddish, apparently semifluid interlaminae. Not infrequently there exists a similar clear reddish boundary layer between ectoplasm and endoplasm (Bütschli).

Endoplasm consisting of colorless or yellowish myxoplasm, usually tinted reddish to reddish-brown (see *Hæmatoidin* below); distinguished from the ectoplasm by its color and by the presence of granules, globules, numerous small nuclei, vacuoles and inclusions (notably hæmatoidin crystals). Granules minute, arranged without order. Globules numerous, irregularly scattered; in all probability fatty, being soluble in alcohol;<sup>1</sup> containing hæmatoidin crystals. The older writers also include the nuclei under the term globules.

Nuclei very numerous, small, with a dark surrounding membrane, granular contents, nucleolus and radiating fibrillae (Bütschli). Pfeiffer remarks<sup>2</sup> that these are to be referred back to the original single nucleus of the young myxosporidium.

Vacuoles (apparently nonpulsating; indefinite as regards number and position), are sometimes seen in forms with few granules.

Hæmatoidin crystals: These were first observed by Lieberkühn.<sup>3</sup> They were subsequently noted by Bütschli,<sup>4</sup> who rightly remarked that they must be derived from the blood of the host; i. e., that they are of extramyxosporidian origin. They occur in the fat globules, and are found free in the protoplasm only after solution of these globules by alcohol. They can be found from the smallest beginnings up to a more conspicuous size, the fat globules then forming a proportionally slight covering for them (Bütschli).

Pfeiffer<sup>5</sup> describes and figures a red blood corpuscle as included within the endoplasm. This he regards as the source of the hæmatoidin crystals. He asserts that they are constantly present and that they occur free or within the fat-globules. He adds that if the myxosporidium has amœboidly surrounded these blood corpuscles and now consumes them, then in spite of the structure of the spores the *Myxosporidia* can no longer be regarded as Gregarines.

Pseudopodia of 2 kinds: (1) Blunt, obtusely rounded, usually formed of ectoplasm alone, endoplasm taking part in formation only where the body as a whole forks. (2) Fine, hair-like or bristle-like, usually rigid, frequently branched, comparable to similar processes of many amœbæ, frequently covering whole surface, not rarely, however, limited to a certain region of same (e. g., the end, as in certain amœbæ);

<sup>1</sup> Bütschli, Bronn's Thier-Reich, 1882, I, p. 594.

<sup>2</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., p. 44.

<sup>3</sup> Müller's Archiv., 1854, p. 350; see also next footnote.

<sup>4</sup> Ztschr. f. wiss. Zool., 1881, XXXV, p. 642; Bronn's Thier-Reich, 1882, I, p. 594. Bütschli credits their discovery to Lieberkühn and Meissner. I infer from Lieberkühn's statement, that Meissner's results were communicated to him orally but were not published.

<sup>5</sup> Die Protozoen als Krankheitserreger, 1890, 1 ed., p. 46; *ib.*, 1892, 2 ed., pp. 17, 132.

both varieties may be retracted and again extruded; some of these processes are, however, optical illusions, being views in optical section of transverse ectoplasmic folds (Bütschli; Pfeiffer).

Amœboid movements<sup>1</sup>: Slow, well seen when examined in the urine of the fish; absent (from rapid death of myxosporidium) in water and many "indifferent" fluids, e. g., egg-albumen solution. Best seen in pike's urine at 24° C.; the ectoplasm executes very extensive amœboid movements, wrinklings, and foldings (Pfeiffer).

*Spore formation.*<sup>2</sup>—Not confined to adult forms, but found in myxosporidia of all sizes. Thus few-spored large, and many-spored small myxosporidia are often seen (Gabriel). This occurrence at different times is explained by successive ripenings of the different individual myxosporidia composing the plasmode. Small round myxosporidia not yet entirely freed from the epithelial cell-remnants often contain 2 or more spores (Pfeiffer).

Pansporoblast formation: This, the first step toward spore formation, takes place by the differentiation within the myxoplasm of a number of small, clear, transparent plasma-spheres (*pansporoblasts*), each consisting of one of the many nuclei of the myxosporidium, together with a portion of the surrounding myxoplasm which it has attracted to it. Sometimes early, and in all cases later, each pansporoblast is surrounded by a thin dark membrane,<sup>3</sup> and is found to contain a number of nuclei, usually 6.

Pansporoblast-segmentation: Subsequently, instead of the pansporoblast consisting, as originally, of the pansporoblast membrane containing a single (usually sexanucleate) plasma-sphere, it comes to consist of the same membrane containing two<sup>4</sup> (usually trinucleate) plasma-

<sup>1</sup> Gabriel (loc. cit.) gives a very detailed description of these movements, concluding that they are so complex and peculiar as to find no parallel with the Gregarines, and none appears admissible with the pseudopodial movements of the *Protozoa*. Special emphasis is placed on the presence in the myxoplasm of a "thread-drawing" (*Fadenziehenden*) substance, capable of emitting pseudopodioid processes, but incapable of retracting them. This, Gabriel asserts, finds a parallel only in myxomycete plasmodes, of which it is an exclusive feature. Bütschli (1881, p. 640) has, however, observed the retraction of these processes.

<sup>2</sup> Description Bütschli's, unless otherwise stated.

<sup>3</sup> Pfeiffer confirms. Upon examining a myxosporidium in a dilute solution of eosin, or other stain, the spores stain only after rupture (by pressure on cover-glass) of this membrane. Gabriel dissents, regarding the pansporoblast as a "wall-less vacuole, which first takes on the vesicular appearance described by Leydig at a later stage." According to Gabriel the pansporoblast does not always persist to maturity, so that in the later stages it may be vainly sought. Gabriel was unable to trace a genetic relation between the "granules" (? nuclei) of the myxosporidium and the spores, whence he concluded that the latter originate by a process, not of myxoplasmic integration but by one of secretion, the morphologic substratum of the sporigenous vacuoles being regarded as *polysporogenetic centers* strongly contrasted with the *monosporogenetic centers* of the Gregarines.

<sup>4</sup> Spores in this species always developed in pairs (Bütschli). Spores not always, though usually, developed in pairs; such paired development may be absent among both developing and free spores (Gabriel).

hemispheres (*sporoblasts, sens. strict.*) which ultimately develop into 2 spores still contained within the pansporoblast membrane.

Development of sporoblast to spore: The fate of the 3 nucleus-like bodies remains in doubt. The central one Bütschli observed to develop into the spore—"nucleus." The other two do not<sup>1</sup> (as would naturally be supposed) develop into the capsules; on the contrary, the 2 nuclei disappear, while the capsules appear in the protoplasm independently of them. Gabriel sometimes observed the sporoblasts (i. e., spores still within the pansporoblast membrane) to undergo a slow progressive contraction to a globular shape, showing their membrane (presumably the future spore-shell) to be not yet rigid. A similar contraction was seen by the same observer in spores with partially disorganized shells.

*Spore*.—Transversely and unequally biconvex-lenticular; length,  $5\ \mu$  ( $\frac{1}{160}''$ , Lieberkühn; 4 to  $6\ \mu$ , Thélohan); breadth,  $20\ \mu$  or less (Bütschli; 15 to  $20\ \mu$ , Thélohan). Shell plainly visible, sharp contoured, rather thick, frequently showing a delicate antero-posterior striation; bivalve structure unknown, sulphuric acid producing no effect. Capsules 1 in each wing<sup>2</sup>; filaments 2 to 3 times the breadth of the spore. Sporoplasm almost completely filling the shell-cavity, extending even to the wings, there surrounding, as a thin layer, the capsules. Nuclei, 2 (*vide* Thélohan, letter 1893). Concerning them and the vacuole-like structure shown in Bütschli's figures, M. Thélohan writes:

The spore of *Myxidium lieberkühni* does not contain a vacuole. This is a fact of which I have assured myself many times. The dark streak shown in Bütschli's figures belongs, without doubt, to the 2 nuclei of the plasmic mass which are often approximated, and, after the action of slightly elective stains, appear blended into a single mass.

*Exit of sporoplasm* (Pfeiffer).—Easily observable by examination of bladder-mucus in urine of pike at  $24^{\circ}\text{C}$ . After 4 to 12 hours a scattered mass of burst shells are seen; also many spores not yet burst, showing the contents much more plainly separated than in fresh specimens. In some individuals the sporoplasm is seen to flow amœboidly out "between the shells" (which are peculiarly unraveled) and wander away.

Gabriel states that during the whole year that he studied this species he never saw the shell split to give exit to the sporoplasm. On the contrary, he describes the process substantially as follows:

Shell undergoing a rather easily observable fluidification or resorption, its contour (heretofore, though thin and delicate, plainly perceptible), after a variable period, entirely disappearing. Sometimes during the resorption stage, always by time of

<sup>1</sup> On the contrary, Pfeiffer (*Die Protozoen als Krankheitserreger*, 1890, 1 ed., p. 98; 1891, 2 ed., p. 132), however, states that the capsules are formed from these 2 nuclei.

<sup>2</sup> Sometimes only 1 capsule at 1 "end," very rarely 2 capsules together in the center (Lieberkühn). Rarely ventricose monstrosities are seen with 2 capsules situated together at 1 "end" (Bütschli). Balbiani figures, beside the usual forms, others with 2 capsules in each wing.

disappearance of shell-contour, significant changes occur, involving capsules as well as sporoplasm, the capsules behaving throughout as integral parts of the "protoplasmic contents." The sporoplasm, previously very transparent, bluish, rather strongly refringent and destitute of granules, becomes paler, sharply contoured granules rapidly appear in spots, and these very delicately contoured, round-elongate or irregular [formerly sporoplasmic, now become myxoplasmic] masses grow slowly or rapidly to small, strongly granulated plasmodes which already show some yellowish or reddish-yellow pigmented spots.

Gabriel has also the following strange statement as to the subsequent course of development:

Now it appears very peculiar that these 3 constantly present, morphologically individualized, delimited, constituent parts [sporoplasm and 2 capsules] should, in their further development, be restricted to a *double course*, viz, either fusing to a single protoplasmic mass or remaining in the original state of separation; in the latter case, falling apart by a rapidly progressing division, each into 2 (rarely more), approximately equal, parts.

*Growth of myxosporidium* (Pfeiffer).—The young myxosporidium [heretofore termed the sporoplasm], immediately after its exit from the spore, penetrates into the interior of the red blood corpuscles and of the cells of the bladder epithelium. The infection of the former may be followed under the microscope. After 8 to 12 hours they show a noteworthy alteration, having become pale and, instead of 1 nucleus, containing 2, 3, or more nuclei. One of these nuclei is *jagged*, or wrinkled; the other (or others) is somewhat smaller, *smooth*, round, shining, and occupies (with reference to the jagged nucleus) a variable position. Hæmatoxylin stains the jagged nucleus dark, the smooth one bright. With the increasing growth of the smooth nucleus the jagged one rapidly falls to pieces, and its remnants become pressed against the cell wall. Methylen blue and phloxin red stain the disrupted jagged nucleus black-blue, the other a uniform red. From these observations and the analogy of *Lacerta* and *Testudo* blood, the jagged nucleus is to be regarded as the cell nucleus, and the smooth nuclei as intruded myxosporidian germs. Here, too, the multiple infection (*Mehrlingsinfektion*) is repeated.

*Microscopic technique*.—Removed from their normal habitat, the myxosporidia rarely remain intact more than 24 hours, and then only in "indifferent" liquids, preferably (besides iodized serum) a 1·5 per cent sodium carbonate solution or a 0·5 per cent sodium chloride solution (Gabriel). Phloxin red and methylen blue stain the ectoplasm a sharply defined red, the entoplasm inclusions blue. This striking result causes the myxosporidium to resemble a true rhizopod (Pfeiffer).

*Habitat and frequency*.—Urinary bladder of *Lucius lucius* (pike). Most frequent and most highly developed in late summer and autumn; rare in winter; thence increasing in frequency. Size and age of host exert no influence (Gabriel). Free-floating in urine or attached (by pronged end). Bütschli observed young examples with one end partly surrounding an epithelial cell which had been torn away, thus presenting a Gregarine-like mode of attachment. Observed by Lieberkühn



attached firmly to *Distoma folium* (frequently found in the pike's bladder); also attached to other myxosporidia. Observed by Bütschli in December.

All individuals of *Lucius* from the Rhine and Saar have myxosporidia in the bladder, while those from the Elbe and Weser territory only exceptionally show them (Pfeiffer, 1891, p. 110).

Perrier erroneously cites the habitat as the *air bladder*.

*Pathology* (Pfeiffer).—The coarser anatomical details can be seen (under 300 or 400 diameters) by carefully stretching a bladder tightly over a cork, placing a cover glass underneath, brief fixation, and hardening by alcohol and staining. Control experiments may be made by maceration in diluted acetic acid. The infection of the bladder was also followed by capillary cultures.

Mucous membrane, when slightly affected, showing individual clusters of 4, 5, 100 or more epithelial cells infected with myxosporidia; thence all grades of hypertrophy (up to 10 to 30 times the normal size) can be traced.

*Hypertrophy of epithelial cells*: When slight, the cells are swollen, shining, apparently lobed. Pfeiffer failed to differentiate the nucleus and the intruder, probably owing to early succumbing of the nucleus. With greater hypertrophy the cells are filled with and overdistended by the parasites; subsequently, continued growth of the myxosporidium ruptures the cell membrane; the myxosporidium flows amœboidly out in grotesque shapes, and immediately differentiates its hyaline ectoplasm; rupture of cell membrane visible under the microscope. Hamatoxylin or phloxinred-methylenblue stains a narrow-bordered, dark globule in the interior of the swollen epithelial cells; nucleus of latter invisible; largest cells indicating, by ragged coloring of contour, the degeneration of the epithelial remains.

*Effects* (of this species??).—Of late years dead pike and perch have frequently floated down the Mosel and the Rhine. It is doubtful whether the disease here is the same as the muscle infection of the barbel. According to a statement [unpublished, I infer] by Dr. T. W. Müller in Greifswald, the spore found in the flesh of the pike is not the same as that of the barbel, but is formed upon the type of *M. lieberkühni* (Pfeiffer).<sup>1</sup>

Whether the pike and perch in the Mosel die from myxosporidiosis is unknown. With the perch, fungus disease concurs (Ludwig).<sup>2</sup>

**101. Myxidium ?? incurvatum** Thélohan, 1892.

Compt. Rend. Acad. Sci. Paris, cxv, pp. 1093-1094.

Cyst, probably none.

*Myxosporidium*.—Small, feebly motile. Ectoplasm (in sections) very clearly striate. Pseudopodia lobed, sometimes forming a bristly, shaggy coat, as in *Myxidium lieberkühni*.

<sup>1</sup> Die Protozoen als Krankheitserreger, 1892, 2 ed., p. 105.

<sup>2</sup> Jahresber. d. rhein. Fisch.-Vereins Bonn, 1888, pp. 27, 28.

*Spore*.—Possessing only one plane of symmetry, viz, the valve-junction plane, differing in this respect from most other myxosporidian spores, which have another such plane perpendicular to valve-junction plane. Form very remarkable, comparable to a pod whose acuminate extremities are oppositely directed; length (?), 4 to 5  $\mu$ ; breadth (?), 8 to 9  $\mu$ . Capsules, 1 at each end (or wing?), their long axes oblique and oppositely directed with reference to the long (transverse?) diameter of the spore. Filament extrusion very difficult of production; produced by nitric acid; length of filament, 12  $\mu$ ; sporoplasm nonvacuolate.

*Habitat*.—Gall bladders of *Onus tricirratus* (= *Motella tricirrata*), *Syngnathus* (= *Entelurus*) *æquoreus* (pipefish), and *Blennius pholis*, all from Roscoff; in *B. pholis* from Concarneau; in *Siphostoma* (= *Syngnathus*) *acus* (pipefish) and *Callionymus lyra*.

The description of this species is not sufficient, in the absence of figures, to warrant a positive opinion as to its generic affinities. I have attempted to construct from Thélohan's description a diagram of the spore, but without success. The prevalent very loose use of such terms as "ends," "extremities," "length," "breadth," etc., renders them invalid for taxonomy, and the only course open seems to be to retain this provisionally in *Myxidium*, noting that in its bivalve structure it differs markedly from *M. lieberkühnii*, the type species.

**102. *Myxidium?* sp. incert. Pl. 47, fig. 6.**

Psorosperms of *Raja batis*, Leydig, 1851, Müller's Archiv., pp. 226, 234, pl. 8, fig.

4g; *ib.*, Leuckart, 1852, Archiv. f. physiolog. Heilkde., XI, p. 436, fig. 21b.

*Myxidium?* sp. Gurley, 1893, Bull. U. S. Fish Com. for 1891, XI, p. 420.

No description. The distinctness of this form from *Chloromyxum incisum* was recognized by Leydig (p. 234).

*Habitat*.—Free in bile ducts of *Raja batis* L. (skate).

## EXPLANATION OF PLATES.

All figures copied are either of the same size as, or  $1\frac{1}{2}$  times the size of, the figures from which they were copied; that is, in copying only 2 ratios were used, 1:1 and  $\frac{3}{2}$ :1. The relative sizes of the copied and the original figures are in every case indicated by the figures *within the parentheses*. All figures outside the parentheses indicate the total amplification from the specimens. For the derivation of any figure, see table, pp. 131-134.

## PLATE 1.

Figs. 1-4. *Psorospermia sciænæ-umbræ* (after Robin.  $\times \frac{3}{2}$ ).

- 1a. The convoluted string (*cordon enroulé*).  $\times 1\frac{1}{2}$ .
- 1b. Section of fig. 1a.  $\times 1\frac{1}{2}$ .
2. Cells of variety 1.  $\times 600$ .
3. Cells of variety 2.  $\times 600$ .
4. Operculate cells of variety 3.  $\times 600$ .

## PLATE 2.

Figs. 1-2. *Lithocystis schneideri* (after Cuénot.  $\times \frac{3}{2}$ ).

1. Gregarine stage, with voluminous nucleus and clinorhombic crystals.
- 2a. Spore at the extremity of the tube, showing the truncated distal and rounded proximal extremities, and the sporozoites in course of formation.
- 2b. Fully developed spore containing 8 sporozoites.

Fig. 3. *Genus incert. sp. 3* (after Müller & Retzius.  $\times \frac{3}{2}$ ). Spores from the diseased air bladder of *Gadus morrhua*.

## PLATE 3.

Figs. 1-5. *Balbiana rileyi* (after Stiles.  $\times 1$ ).

1. A portion of the pectoral muscles of *Anas boschas* in the condition known as "measly duck."
2. Longitudinal section of parasite (greatly enlarged).
3. Transverse section (greatly enlarged): *ct*, connective tissue cyst with numerous nuclei; *cu*, cuticle of the parasite; *m*, sections of muscle.
4. Microtome section of meshes containing falciform bodies greatly enlarged.
5. Falciform bodies: *a*, stained, showing nucleus and vacuole; *b*, unstained.

## PLATE 4.

Fig. 1a-m. *Genus incert. sp. 4* (after Valentin.  $\times \frac{3}{2}$ ).

- 1a. The original globular form.
- 1b-d. Different stages of the unrolling of the tail.
- 1e. A globule in which the separate dark granules appear to be inclosed in separate peduncles.
- 1f. Peduncle ideally enlarged.
- 1g-m. Various forms of the developed animal.

Figs. 2-8. *Genus incert. sp. 6* (after Schewiakoff.  $\times 1$ ).

2. Amœbiform stage.  $\times 1500$ .
- 3-5. Encystment.  $\times 1500$ .
6. Cyst with 6 spores.  $\times 1500$ .
7. Cyst thickly filled with spores.  $\times 1500$ .
8. Plasmode proceeding from the fusion of 3 amœbæ.  $\times 1500$ .

## PLATE 5.

- Figs. 1-11. *Genus incert. sp. 6* (after Schewiakoff.  $\times 1$ ).  
 1-3. Developmental stages of the plasmode.  $\times 1500$ .  
 4. Encystment.  $\times 1500$ .  
 5. Cyst-tube with spores.  $\times 1500$ .  
 6. A ruptured cyst with emerging spores.  $\times 1500$ .  
 7. Spores sessile on the muscles.  $\times 1500$ .  
 8. Individual spore.  $\times 2600$ .  
 9. Small plasmatic corpuscles proceeding from the spores.  $\times 2600$ .  
 10a-l. Transverse division of the spore; the nucleus dividing karyokinetically.  $\times 2600$ .  
 11a-b. Conjectural conjugation stages of the spores.  $\times 2600$ .

## PLATE 6.

- Fig. 1. *Genus incert. sp. 9* (after Lieberkühn in Bütschli.  $\times \frac{3}{2}$ ).  $\times$  about 195.  
 Myxosporidium from the gall bladder of *Lota lota*.  
 Fig. 2. *Genus incert. sp. 10* (after Lieberkühn in Bütschli.  $\times \frac{3}{2}$ ).  $\times$  about 195.  
 Myxosporidium from branchiæ of *Lota lota* with a very thick ectoplasm.  
 Figs. 3-8. *Genus incert. ("Myxosporidium") congrei* (after Perugia.  $\times 1$ ).  
 3-4. Two forms with "vacuoles."  
 6. An individual attached to a vegetable filament.

## PLATE 7.

- Figs. 1-3. *Genus incert. sp. 12* (after Linton.  $\times 1$ ).  
 1. *Notropis megalops* with dermal cysts caused by "psorosperms."  $\times 1\frac{1}{2}$ .  
 2. Spores from cysts, highly magnified.  
 2a. Vertical view of spores in caustic potash.  
 2a'. Same, more highly magnified.  
 2b. Transverse view of spore.  
 2b'. Same, more highly magnified.  
 2c. Spore treated with sulphuric acid.  
 3. Portion of thin section of cyst: a, pigment spot; b, granular protoplasm; c, spores; d, wall of cyst and dermis.  $\times$  about 150.  
 Fig. 4. *Genus incert. sp. 13* (after Lieberkühn.  $\times \frac{3}{2}$ ).  
 4a. Spores from a subcutaneous cyst of *Gasterosteus aculeatus*.  $\times 870$ .  
 4b-e. The same in different stages of development; b, spore with plain "nucleus" of usual size; c, d, with smaller "nucleus;" e, "nucleus" scarcely perceptible, the previously plain membrane no longer visible, animal mature.  
 Fig. 5. Sarcosporidian spore of sheep with a "capsule" (after Pfeiffer.  $\times 1$ ).

## PLATE 8

- Figs. 1-4. *Genus incert. ("Myxosporidium") bryozoides* (after Korotneff.  $\times 1$ ).  
 1. Filament of *Alcyonella fungosa*, with the spermatozooids and the parasite on them.  $\times 350$ .  
 2. A parasite inclosed in an *Alcyonella* zooid.  $\times 350$ .  
 3, 4. Creeping adults with nuclei and spores.  $\times 750$ .

## PLATE 9.

- Figs. 1-4. *Genus incert. ("Myxosporidium") bryozoides* (after Korotneff.  $\times 1$ ).  
 1a. Group of spermatoblasts, 2 of them containing very young stages of the parasite.  $\times 900$ .  
 1b-d. Different stages in the conversion of a spermatoblast into a plasmode; cell nuclei and parasite nuclei shown.  $\times 900$ .  
 1e. Plasmode in which 1 daughter, and 2 granddaughter cell nuclei are visible. Nuclei of parasite numerous.  $\times 900$ .  
 2. A plasmode in which the cell nuclei are atrophying and possess a jagged contour.  $\times 900$ .  
 3. Spores in which vacuoles and urticant organs are to be distinguished.  $\times 900$ .  
 4. Nuclei of the parasite of plate 8, fig. 3.

## PLATE 10.

Figs. 1-3. *Glugea anomala*.1a-h. (After Gluge.  $\times 1$ .)1a, b. Showing *Gasterosteus aculeatus* with tumors on sides of body and on tail.  $\times 1$ .1c-e. Spores variously magnified.  $\times 255-840$ .

1f, g. The same "coagulated."

1h. Cyst membrane.

2. Section showing, from above downward, subcutaneous connective tissue, cyst membrane, protoplasmic contents of cyst, and spores (after Thélohan.  $\times 1$ ).3a-i. Group of spores: a, b, fresh; c-i, safranin stained; c, d, spores with 1 nucleus; e, f, with 2 nuclei; g, with 3; h, i, with 4 (after Thélohan.  $\times 1$ ).Figs. 4-5. *Thelohania contejeani* (after Henneguy and Thélohan).4. Longitudinal section of diseased crayfish muscle ( $\times 1$ ).5a. Spores in sporophorous vesicle, and free ( $\times \frac{3}{2}$ ).5b. Individual spore, more highly magnified ( $\times \frac{3}{2}$ ).Fig. 6. *Thelohania octospora* (after Henneguy.  $\times 1$ ).

6a. Sporophorous vesicle with spores.

6b. Individual spores.

6c. Longitudinal section of diseased muscle of *Palmon rectirostris*, showing sporophorous vesicles between the separated fibrillæ.

6d. Portion of c more highly magnified.

## PLATE 11.

Figs. 1-5. *Thelohania octospora* (after Henneguy and Thélohan.  $\times 1$  except fig. 5).1. Transverse section of entire abdomen of a badly diseased *Palmon rectirostris*, showing, opposite the letters, the following: m, m, affected muscles; dt, digestive tube; n, nerve cord; cl, sections of the claws.

2. Longitudinal section of muscle showing the dissociation of the fibrillæ.

3. Transverse section of diseased muscle.

4. A part of fig. 2, more highly magnified, showing fibrillæ with very clear striation, and the sporophorous vesicles.

5a-d. Showing the spores: b, in the fresh state showing the vacuole; a, c, d, after action of ether; a, with the filament partially, c and d with it completely, extruded ( $\times \frac{3}{2}$ ).

## PLATE 12.

Figs. 1-2. *Thelohania giardi* (after Henneguy and Thélohan).1. Spore formation ( $\times \frac{3}{2}$ ).

1a. Young pansporoblast.

1b. Pansporoblast whose nucleus has lost its membrane and presents itself under the form of an equatorial plate.

1c. Pansporoblast whose nucleus has segmented into 2.

1d. Pansporoblast the protoplasm of which has segmented into 2 uninucleate plasma hemispheres.

1e. Pansporoblast in the IV stage; fresh state.

1f. Pansporoblast in the IV stage, the augmentation of size of nuclei and change in disposition of chromatin preliminary to division.

1g. Pansporoblast in the IV stage; nucleus in repose.

1h, i. Pansporoblast in the VIII stage; different dispositions of the sporoblasts (the 8th in i is not represented, being hidden by the others).

1k. Sporophorous vesicle inclosing 8 ripe spores.

1l. Pansporoblast inclosing 4 normal spores, and 2 bodies each formed by the soldering together of 2 spores by their large ends: a, thickening of the pansporoblast membrane; b, spores soldered; s, normal spores.

1m, n. 2 sporoblasts with crescentic nucleus. In the concavity of the latter, a clear vacuole. At n a small protoplasmic button projects into the vacuole.

1o. Spores in fresh state showing at the large end a clear vacuole and at the small, a brilliant point corresponding to the capsule.

1p. Spores showing the vacuole and the longitudinal shell-striæ.

1q, r. Spores after action of sulphuric acid: q, filament incompletely unrolled; r, filament completely unrolled.

## PLATE 12—Continued.

- Fig. 2. Pathological anatomy ( $\times 1$ ). Longitudinal section of diseased muscle of *Crangon vulgaris*, showing fibrillæ with normal aspect preserved, and pansporoblasts in different stages of development, and spores.
- Fig. 3. *Thelohania macrocystis* (after Garbini.  $\times 1$ ).
- 3a-c. Sporophorous vesicle and spores.
- 3d. Spores.
- 3e. A section of the diseased tissue.

## PLATE 15

- Fig. 1. *Myxobolus unicapsulatus* (after Müller.  $\times 1$ ).
- 1a, b. Vertical view of spores, showing the single capsule and the sporoplasm.
- 1c. Vertical view of spore, showing sporoplasm (and vacuole?).
- 1d. Transverse view of spores.
- Fig. 2. *Myxobolus inequalis* (after Müller.  $\times 1$ ).
- 2a. Vertical view, showing the unequal capsules and the sporoplasm.
- 2b. Transverse view.
- Fig. 3. *Myxobolus piriformis* and *M. ellipsoides*. Spores highly magnified from Malpighian corpuscles of spleen of *Tinca tinca* (after Balbiani.  $\times 1$ ).
- 3A. Nos. 1, 2, 6, *Myxobolus piriformis*? (see p. 211, footnote 1), showing the elongate pyriform outline and the single capsule.
- Nos. 3, 4, 5, 7, *Myxobolus ellipsoides*? (see p. 211, footnote 1).
- 3B, C. *Myxobolus piriformis* or *M. ellipsoides* (which?).
- Fig. 4. "Degenerated forms" from the spleen, liver, and kidney of *Tinca tinca* (after Balbiani.  $\times \frac{1}{2}$ ).
- 4a. *Myxobolus ellipsoides*? (see p. 211).
- 4b, c. *Myxobolus piriformis* (see p. 211).
- 4d-f. *Myxobolus piriformis* or *M. ellipsoides* (which?).

## PLATE 14.

- Figs. 1-3. *Myxobolus brachycystis* (after Remak).
1. Pigment follicle from spleen of *Tinca tinca*, containing 3 "vesicles" [pansporoblasts], each with a pyriform spore. To the right some of the pigment-containing vesicles which fill the cyst. (All *vide* Remak.  $\times 1$ .)  $\times 200$ .
2. Three oval vesicles with pyriform spores from the kidney of *T. tinca* ( $\times \frac{1}{2}$ ).  $\times 375$ .
- 2a. Showing spores and numerous pigment cells.
- 2b. Showing 2 smaller vesicles, each with a pyriform spore.
- 2c. A vesicle showing conspicuous thickenings of its wall.
3. Vertical view of 2 pyriform spores with 2 capsules from tubiform cysts of the spleen of *T. tinca*. Similar spores are also found on the branchiæ and in the kidneys. (All *vide* Remak.  $\times \frac{1}{2}$ .)  $\times 675$ .
- Fig. 4a-g. *Myxobolus*? sp. 38 (after Lieberkühn.  $\times \frac{1}{2}$ ).  $\times 675$ .
- 4a. Vertical view of spore.
- 4b-d. Spore in act of giving exit to sporoplasm.
- 4e-g. Free sporoplasmata of spores.
- Figs. 5, 6. *Myxobolus*? *mugilis* (after Perugia.  $\times 1$ ).
5. Branchial lamella of *Mugil auratus* with cysts.
6. Vertical view of spore.
- Fig. 7. *Myxobolus* sp. 40 (after Lieberkühn in Bütschli.  $\times \frac{1}{2}$ ).  $\times$  about 1050.
- 7a. Vertical view.
- 7b. Transverse view.
- Figs. 8a-d. *Myxobolus oviformis*. From cyst of fins of *Gobio gobio*; safranin and gentian violet (after Thélohan.  $\times 1$ ).
- 8b. Vertical view of spore showing 1 nucleus.
- 8c. Same, with 2 nuclei.
- 8d. Same, with 3 nuclei.

## PLATE 15.

Figs. 1-6. *Myxobolus?* sp. 41 (after Lieberkühn; except 1).

1. Two spores inclosed in the pansporoblast membrane (after Bütschli.  $\times \frac{3}{2}$ ).  $\times$  about 1050.
2. Cyst from branchiæ of *Gasterosteus aculeatus* ( $\times 1$ ).
3. Free spores from cyst of fig. 2. ( $\times \frac{3}{2}$ ).  $\times 675$ .
4. Another cyst in which spore formation has taken place ( $\times 1$ ).  $\times 330$ .
5. Another cyst ( $\times 1$ ).  $\times 220$ .

6a-c. "Different forms [? developmental stages] of spores" of this species ( $\times \frac{3}{2}$ ).

Fig. 7a-c. *Myxobolus* sp. 44.

- 7a. Transverse view of spore (after Lieberkühn in Bütschli.  $\times \frac{3}{2}$ ).  $\times 1350$ .
- 7b. Spore with valves separating, giving exit to sporoplasm (after Lieberkühn.  $\times \frac{3}{2}$ ).  $\times 1350$ .
- 7c. Sporoplasm undergoing amœboid movements (after Lieberkühn.  $\times \frac{3}{2}$ ).  $\times 1350$ .

## PLATE 16.

Figs. 1-6. *Myxobolus mülleri* (after Bütschli.  $\times 1$ , except fig. 1).

1. Two branchial lamellæ of a cyprinoid, one containing a conspicuous myxosporidium. c. The cartilaginous rod supporting the lamella ( $\times \frac{3}{2}$ ).
2. A portion of the membrane of fig. 4, more strongly magnified, showing "nuclei."
- 3a. Transverse view of spore.
- 3b. Transverse view of 2 separated valves.
4. An isolated small myxosporidium with its membrane.
5. Nuclei of the myxosporidium.
6. A series showing the developmental stages of the spore.
- 6a. Sporoblast which has segmented into the 2 protocysts and the protosporoplasm.
- b-c. The segments have oriented themselves; the protocysts show beginning capsule formation.
- d, e. Later stages of capsule formation. In e orientation of the capsules has taken place.

## PLATE 17.

Figs. 1-7. *Myxobolus mülleri* (after Bütschli.  $\times 1$ ).

- 1a. Vertical view; showing capsules, sporoplasm, vacuole and pericorncal nuclei.
- 1b. Vertical view; showing capsules, "globules," sporoplasm, and vacuole.
- 1c. Vertical view, showing a common focus-appearance (?focus-illusion), the pericorncal nuclei apparently attached to the posterior extremity of the capsules. Bütschli says the sporoplasm is "contracted" and hence the vacuole is invisible.
2. Transverse view of spore after action of concentrated sulphuric acid; the filaments are extruded and the valves are beginning to gape apart.
3. Vertical view of spore with extruded filaments, sporoplasm, and "globules."
- 4a-d. "Abnormal" tailed spores; c, spore with 3 capsules.
5. A separated valve, viewed transversely.
6. Spore with filaments extruded by pressure.
- 7a. Capsule not yet completely developed, with the filament extruded.
- 7b. A fully-developed capsule with extruded filament.

## PLATE 18.

Figs. 1, 2. *Myxobolus piriformis* and *M. ellipsoides* (after Balbiani.  $\times 1$ ).

1. Section of splenic artery of *Tinca tinca*, showing on the branches Malpighian corpuscles, most of them containing *Myxosporidia*.
2. The same, more highly magnified, showing well-developed bicapsulate forms (*M. ellipsoides*) and pyriform unicapsulate or noncapsulate and degenerate forms (*M. piriformis*).

## PLATE 19.

Fig. 1. *Myxobolus bicostatus* (after Lieberkühn in Bütschli.  $\times \frac{3}{2}$ ). Vertical view of spore showing the 2 oblique ridges on the shell, the capsules, and the sporoplasm.

Figs. 2-8. *Myxobolus ellipsoides*.

2, 3. Pfeiffer's copies of figs. 1a, 1b of plate 20 ( $\times 1$ ).

4. Mesenteric artery of *Tinca tinca* with sessile or pedunculate cysts developed at the expense of the connective tissue coat of the vessel. Cyst contents myxosporidia, alone or with imbedded brown (hæmatoidin-colored) granular matter (after Balbiani.  $\times 1$ ).

5. Section of diseased air bladder of *T. tinca*, showing spores and, at the left-hand margin, the internal epithelial surface of the air bladder. Borax carmine, gentian violet (after Thélohan.  $\times 1$ ).

6. Section of cyst of branchia of *T. tinca*; showing in order, from above downward, the branchial epithelium, cyst membrane, myxoplasm, spores, and the nuclei of the last. Picro-carmine and gentian violet (after Thélohan.  $\times 1$ ).

7. Transverse section of air bladder; carmine, celloidin (after Pfeiffer.  $\times 1$ ).  $\times 100$ .

8. Portion of fig. 7 (after Pfeiffer.  $\times 1$ ).  $\times 400$ . On the wall of the cyst the younger, still uninuclear, parasites; to the right trinucleate sporoblasts.

## PLATE 20.

Figs. 1-4. *Myxobolus ellipsoides*.

1a-c. Myxosporidium and cyst from fins of *Tinca tinca*, with spores in course of development (after Balbiani.  $\times 1$ ).

1a. Small myxosporidium containing only nuclei.

1b. More advanced stage.

1c. Large encysted myxosporidium containing numerous spores, mostly mature.

2a-c. Three stages in spore formation, showing paired development of spores in a mass of homogeneous plasma, and the spores contained at maturity in a vesicle (after Balbiani.  $\times \frac{3}{2}$ ).

3a-c. Spores from air bladder of *T. tinca* showing ribbons (after Balbiani.  $\times \frac{3}{2}$ ).

3a, b. Spores united by the ribbons, the sporoplasm rolled into a ball, and the "accessory" capsules.

3c. Isolated spore with extended ribbons; capsules empty; sporoplasm in a ball.

4a-e. Spores from the air bladder of *T. tinca*, showing different stages of development of the nuclei; carmine, gentian violet (after Thélohan.  $\times 1$ ).

4a. Spore with 1 nucleus.

4b. Spore with 2 nuclei.

4c. Spore with 3 nuclei.

4d, e. Spores with 4 nuclei.

## PLATE 21.

Figs. 1, 2. *Myxobolus ellipsoides*.

1a-h. (After Balbiani.  $\times \frac{3}{2}$ .)

1a. Vertical view of spore, showing pericornual nuclei and anteriorly a "globule."

1b. Transverse view, showing the equal convexity of the valves and the equality of the two ends of the spore.

1c. Vertical view of spore, showing capsules with filaments extruded, pericornual nuclei, anteriorly a "globule," and posteriorly the sporoplasm (? contracted under the action of reagents).

1d. Spore in vertical view, showing ribbons, and sporoplasm in act of exit.

1e. Capsule with filament coiled.

1f-h. Different degrees of extrusion of filament.

2a-e. Sporoplasm after exit, showing changes of form (after Balbiani.  $\times \frac{3}{2}$ ). n, "nucleus" [? vacuole].

Fig. 3. "Degenerate processes of the spores of *Tinca tinca* with 3, with 2 approximated, with 1 capsule, with caudiform drawing out of one pole, with approximation to the sarcosporidian germs. The same are found in the gall bladder of the tench and in aneurisms on the splenic artery" (after Pfeiffer.  $\times 1$ ).  $\times 1000$ .

d. *Myxobolus ellipsoides* (apparently; remainder indeterminate).

Fig. 4a-b. *Myxobolus ellipsoides*?? "Spores inclosed in a cell [? pansporoblast] membrane becoming stained at the moment of birth, with eosin" (after and *vide* Pfeiffer.  $\times 1$ ).  $\times 750$ .

Fig. 5. *Myxobolus ellipsoides*. "Mature spore, with band-like connection of shell, and with vacuole at place of expelled germ" (after and *vide* Pfeiffer.  $\times 1$ ).  $\times 750$ .



## PLATE 22.

Figs. 1-3. *Myxobolus ellipsoides*? (after Pfeiffer.  $\times 1$ ).

1, 2. Spores from the gall bladder of *Tinca tinca*.

3. Spores from the air bladder of *T. tinca*.

Fig. 4a, b. *Myxobolus* sp. 50 (after Leuckart.  $\times 1$ ).

4a. Vertical view of spore.

4b. Transverse view of spore.

Figs. 5, 6. *Myxobolus* sp. 51 (after Pfeiffer.  $\times 1$ ).

5. Myxosporidian infection of *Barbus barbus*.

6. Tumors of muscle.

## PLATE 23.

Figs. 1-2. *Myxobolus* sp. 51. Myxosporidian infection of *Barbus barbus* (after Pfeiffer.  $\times 1$ ).

1. From a photomicrograph.

2. Infection of the muscle cells and the interfibrillar connective tissue.

2a. General immigration of myxosporidian spores into muscle with degeneration of the neighboring parts of the muscle and with beginning of incapsulating on the part of the host.

2b. Split spores. To the left, the exit of the sporoplasm; to the right, empty shells undergoing solution.

2c. The myxosporidium (sporoplasm) in the first stages of growth; on the right the same, after hardening and hematoxylin.

2d. Next growth-stage of myxosporidium; adhesion of individuals to a "sorus."

## PLATE 24.

Figs. 1, 2. *Myxobolus* sp. 51 (after Pfeiffer.  $\times 1$ ).

1a-h. Sections of muscles of *Barbus barbus*, showing myxosporidian cysts, spores, etc. For details, see Bibliography, LXXII. p. 127.

2a. A large muscle cell of abdominal wall beaded by myxosporidian cysts; the transverse striation and the substance of the muscle has disappeared. Size of cysts, variable; contents, spores.  $\times 100$ .

2b. Fragment of muscle cell. Showing 5 spore cysts. Between the upper and the next to the upper cysts lie 7 spores in the muscle cell (supplementary immigration?).  $\times 100$ .

2c. Fragment of another muscle cell with 6 cysts. The upper 2 with mature spores; between them 6 spores, whose capsules lack the oblique striation (filaments extruded?). The third cyst with the contents divided into pansporoblasts, in which as yet no spores are visible. The fourth and fifth (from above) showing nuclei, surrounding dancing granules, and a hyaline ectoplasm; both are inclosed in a mesh of the original muscle cell.  $\times 400$ .

2d. Myxosporidium free in the interfibrillar connective tissue.  $\times 750$ .

2e. Mature spore.  $\times 750$ .

## PLATE 25.

Figs 1-6. *Myxobolus* sp. 51.

1 a-h. Group of spores, most of them viewed vertically (after Ménézin.  $\times \frac{1}{2}$ ).

1b. Spore with filaments extruded.

1c. Isolated capsules.

1d. Same, with extruded filament.

1e. Spores viewed transversely.

1f-h. Spores apparently imbedded in the myxosporidium.

2. Showing a, vertical, and b, transverse views of spore, and c, a transverse view of a separated valve (after Ludwig.  $\times 1$ ).  $\times 2000$ .

3. Spore viewed vertically (after Pfeiffer.  $\times 1$ ).

4. Isolated myxosporidium, showing spore formation (after Pfeiffer.  $\times 1$ ).

5. Spores and the extruded amœboid sporoplasm (after Pfeiffer.  $\times 1$ ).

5a. Vertical view, showing one capsule with filament extruded, sporoplasm, vacuole, and 3 refringent bodies of undetermined significance.

5b. Transverse view of spore showing ridge.

5c. Sporoplasm, after exit, in various locomotive stages.

6. Spores, showing filaments extruded, and sporoplasm in the act of, and after exit, apparently also the vacuole (after Pfeiffer.  $\times 1$ ).  $\times 1500$ .

## PLATE 26.

Fig. 1. *Myxobolus* sp. 52. Section of a pigment follicle of the walls of the splenic artery; after slight pressure the pigment globules are seen showing untailled spores (after Remak.  $\times 1$ ).  $\times 200$ .

Fig. 2. *Myxobolus* sp. 53 (after Rayer.  $\times 1$ ). Vertical views of spores.

Figs. 3-6. *Myxobolus oblongus*.

3. Branchiæ with cysts (after Müller.  $\times 1$ ).

4. Individual lamellæ with cysts (after Müller.  $\times 1$ ).

5. Spores (after Müller.  $\times 1$ ).

5a. Vertical view.

5b. Transverse view.

6. Spores (original).

6a. Broadest form, showing, in the sporoplasm, the central tongue-shaped dark-staining portion and the first and third series of nucleiform bodies. Gentian violet; slightly diagrammatic.

6b. More elongate form, showing the tongue-like process and the second and third series of nucleiform bodies. Gentian violet; somewhat diagrammatic.

6c. Narrower form, showing the first and second series of nucleiform bodies. Hydrochloric acid alcohol carmine.

6d. Narrow form, showing the 3 series of nucleiform bodies and posteriorly an unusual appearance. Hydrochloric acid alcohol carmine.

6e. Transverse view of spore, showing equality of valves and relative width of ridge (ridge index).

Figs. 7-8. *Myxobolus lintoni* (original). Vertical views of 2 spores, showing capsules and sporoplasm, the latter with vacuole and 4 nuclei (2 of them the pericornual). Hydrochloric acid alcohol carmine.

## PLATE 27.

*Myxobolus lintoni* (after Linton.  $\times 1$ ). Nos. 2-13, highly magnified.

1. *Cyprinodon variegatus*, with excrescences caused by this species; one on right side, and another on left side showing above outline of back.  $\times 1\frac{1}{2}$ .

2-3. Spores showing the pericornual nuclei. In fig. 3 there are a few small refractile globular masses near the posterior end.

4. Spore treated with osmic acid, showing mouths of the ducts.

5-6. Spores in transverse view, showing the ridge.

7. Spore treated with acetic acid, showing vacuole (exaggerated).

8. Diagram of cross-section, showing lenticular shape of spore.

9-11. Specimens treated with concentrated sulphuric acid.

9. With a few refractile bodies and 1 filament extruded.

10. Spore with both filaments extruded and a number of small refractile globules.

11. Spore with sporoplasm "contracted" [? shrunk by reagents]; "a thread also appears at the end opposite the polar vesicles."

12-13. Free capsules and filaments, after treatment with concentrated sulphuric acid.

14. Calcareous bodies found in the abnormal tissue, associated with the *M. lintoni*.  $\times 200$ .

15. Three of the same, with a few spores. Sketch from material after action of potassic hydrate.  $\times 400$ .

16. Spores *in situ*: (a) nests of spores; (b) section of blood capillary; (c) connective tissue. Sketch made from a section of decalcified abnormal tissue.

## PLATE 28.

Figs. 1-3. *Myxobolus globosus* (original).

1a. Vertical view of spore, showing capsules and sporoplasm, the latter containing a vacuole and 4 nuclei, 2 of them being the pericornual.

1b. Transverse view of spore, showing the equal convexity of the valves and the wide ridge.

2, 3. Vertical views of spores exhibiting the same features as fig. 1a.

Fig. 4. *Myxobolus* sp. 56 (original). Vertical views of spores, showing capsules and sporoplasm, the latter with the vacuole.

## PLATE 28—Continued.

- Fig. 5.\* *Myxobolus cycloides* (after Müller.  $\times 1$ ).  $\times 1$ .  
 5a. Group of cysts, natural size.  
 5d. Vertical view of broad form.  
 5e. Transverse view of same.  
 5f. Vertical view of elongate form.  
 5g. Transverse view of same.  
 Fig. 6. *Myxobolus* sp. 61 (after Müller.  $\times 1$ ).  
 6a. Vertical view of spores.  
 6b. Transverse view.  
 6c. Rare aberrant form among the remaining normal forms in the same cyst.  
 6d. Pansporoblasts with 2 spores.  
 6e. Rare forms of pansporoblast containing 3 spores.  
 6f. A rare method of grouping of 3 spores.  
 6g. Spores with punctate borders [illusion due to the simultaneous presence in (approximate) focus of the supero-anterior and infero-anterior borders of the sporoplasm].  
 6h. Spore with developing germs (see p. 240).  
 6i, k. Rare spores with 3 "vesicles."  
 6l. Rare form; seen only once.  
 Fig. 7. *Myxobolus obesus* (after Balbiani.  $\times \frac{2}{3}$ ).  
 7a. Vertical view of spore, showing pericornual nuclei.  
 7b. Vertical view of spore, showing capsules with filaments extruded, and the sporoplasm with its cornua, and the supero- and infero-anterior margins.

## PLATE 29.

- Fig. 1a-d. *Myxobolus transoralis* (original).  
 1a-c. Vertical view showing outline, capsules, sporoplasm, vacuole, and nuclei. Hydrochloric acid alcohol carmine.  
 1d. Transverse view showing equal convexity of valves, and the narrow ridge.  
 Figs. 2-7. *Myxobolus?* *merlucii* (after Perugia.  $\times 1$ ).  
 2-6. Various forms of the myxosporidium; showing also the spores.  
 7. Two spores making their exit from the myxosporidium.  
 Fig. 8. *Myxobolus* sp. 67 (after v. d. Borne.  $\times 1$ ).  
 8a. Group of spores.  
 8b. *Leuciscus rutilus* with the myxosporidian tumors.

## PLATE 30.

- Fig. 1a-g. *Myxobolus* cf. *creplini* showing different views of spores (after Weltner.  $\times 1$ ). a-p,  $\times 528$ ; g,  $\times 720$ . All were drawn with Abbe camera; m, n, are optical sections at the level of posterior end of capsules; g, separate capsules; one dull and with filament still coiled; the other transparent with filament extruded.

## PLATE 31.

- Fig. 1a-c. *Myxobolus* ?? *zschokkei* (after Zschokke, Schieck Oc. 2, Obj. 7.  $\times \frac{2}{3}$ ). Vertical views of spores with extruded "tails"; also the capsules (?).  
 Fig. 2. *Myxobolus medius* and *Chloromyxum elegans*. Section of tube of kidney of *Pygosteus pungitius*, showing spores of the two species surrounded by epithelium of tube. Borax carmine and gentian violet (after Thélohan.  $\times 1$ ).  
 Fig. 3. *Myxobolus medius* (original enlargement from preceding.  $\times$  about 4).  
 Fig. 4. *Myxobolus medius*. Spore in pansporoblast (after Thélohan.  $\times 1$ ).  
 Fig. 5. *Myxobolus strongylurus* (after Müller.  $\times 1$ ).  
 5a. Vertical view.  
 5b. Transverse view.

\* For b and c, see *Chloromyxum dujardini*, plate 40, fig. 4.

## PLATE 32.

Figs. 1, 2. *Myxobolus creplini*.1a-e. (After Creplin.  $\times 1$ .)

1a, b. Vertical view of spores.

1c. Transverse view.

1d. Vertical view (of an illusory appearance? See p. 249). The larger size of this figure merely represents higher magnification.

1e. Transverse view of spore with the valves gaping anteriorly.

2. Vertical view of spore (after Leuckart.  $\times 1$ ).Figs. 3, 4. *Myxobolus monurus* (after Ryder.  $\times 3$ ).3a. *Aphredoderus sayanus* with tumors.

3b. Cyst, much enlarged.

3c. Vertical views of 2 spores, showing capsules and tails.

4b-d. \* Vertical views of spores.

Fig. 5. *Myxobolus macrurus* (original). Vertical view of spore, showing capsules, sporoplasm with vacuole and 3 nuclei (2 the pericornual), and the full length of the tail (about 4 times that of the body).

## PLATE 33.

Figs. 1-4. *Myxobolus macrurus* (original).

1. Transverse view showing, on the right side, the more convex superior valve and the greater anterior projection of the supero-medial cornu; on the left, the less convex inferior valve; along the center, the narrow ridge.

2. Vertical view, showing the vacuole and nuclei.

3. The same, showing also the beading of the tail after the action of iodine.

4. A tail separated from the body by iodine.

Figs. 5-8. *Myxobolus cf. linearis* (original).

5. Vertical view, showing divergence of valves under action of sulphuric acid, and the tail separating into a superior and an inferior half.

6. Transverse view, showing supero-inferior symmetry and narrow ridge.

7. Vertical view of unstained spore, showing vacuole.

8a-d. Vertical views of spores, showing vacuole, nuclei, and flexibility of tail. Hydrochloric acid alcohol carmine.

## PLATE 34

Figs. 1-4. *Myxobolus psorospermicus*.1. From branchiae of *Perca fluviatilis* (after Lieberkühn in Bütschli.  $\times \frac{3}{2}$ ).  $\times$  about 975.

1a. Vertical view of spore with a simple tail.

1b. Transverse view of same.

1c. Vertical view of spore with a double tail.

2a-c. From a branchial cyst of *P. fluviatilis*, showing capsules, sporoplasm, vacuole, and nuclei. a, with 1 nucleus; b, with 2 nuclei; c, with 3 nuclei.Carmine and gentian violet (after Thélohan.  $\times 1$ ).3a-d. Spores from *Perca fluviatilis* (after Balbiani.  $\times \frac{3}{2}$ ).

3a. Vertical view.

3b. Transverse view of spore with 2 tails.

3c. Form slightly abnormal.

3d. Vertical view of spore, showing capsule with filaments extruded, cornua of sporoplasm, and pericornual nuclei.

4. Spores from *Lucius lucius* (after Balbiani.  $\times \frac{3}{2}$ ).

4a. Vertical view.

4b. Transverse view.

4c. Spore with valves separating to permit exit of sporoplasm

4d. Vertical view showing filaments extruded, and cornua of sporoplasm.

## PLATE 35.

Figs. 1-7. *Myxobolus kolesnikovi* (after Kolesnikoff).1-6. Cysts ( $\times 1$ ).7a-o. Spores showing extruded filaments and single and double tails ( $\times \frac{3}{2}$ ).

7g. Separated capsule with extruded filament.

\*No a to this figure.

## PLATE 36.

- Fig. 1. *Myxobolus schizurus* (after Müller.  $\times 1$ ).  
 1a. Showing cyst contents, consisting of spores and finely granular matter.  
 1b. Individual spores.  
 1c. Aberrant spores seen only once among the contents of a cyst.  
 1d. Group of spores; vertical and transverse views.  
 Fig. 2. *Myxobolus linearis*. Group of spores showing the narrow outline and the single and double tails (after Müller.  $\times 1$ ).  
 Fig. 3. *Myxobolus* sp. 61. Rare forms of spores reproduced among tailed forms, from plate 28, fig. 6.  
 Fig. 4. *Myxobolus diplurus* (after Lieberkühn in Bütschli.  $\times \frac{3}{2}$ ).  $\times$  about 1050. Vertical view showing posterior position of capsules and double tail.

## PLATE 37.

- Fig. 1 a-f. *Chloromyxum incisum* (after Leydig.  $\times 1$ ).  
 1a. Myxosporidium without pansporoblasts.  
 1b. Same with 1 pansporoblast, but no spores.  
 1c, d. Same with sporoblasts.  
 1e, f. Same with fully developed spores showing the crenate posterior border.  
 Fig. 2-7. *Chloromyxum leydigii* (after Perugia.  $\times 1$ ).  
 2. The myxosporidium.  
 3. The same, containing numerous spores.  
 4. The same, giving exit to 3 monosporophorous pansporoblasts.  
 5, 6. Pansporoblasts with spores; in fig. 5 the spores with 4 capsules.  
 7. Spore giving exit to the sporoplasm.

## PLATE 38.

- Figs. 1, 2. *Chloromyxum leydigii* (after Leydig.  $\times \frac{3}{2}$ ).  
 1. From gall bladder of *Raja batis*.  
 1a<sub>1</sub>, a<sub>2</sub>. Myxosporidia of various sizes without pansporoblasts.  
 1b, e. Myxosporidia, showing (b) pansporoblasts and various stages in spore formation; also outline of spore.  
 1f. Longitudinal ("end") view of spore, showing the 4 capsules.  
 2a-c. From gall bladder of *Squalus acanthias*. Myxosporidia without pansporoblasts.

## PLATE 39.

- Figs. 1-3. *Chloromyxum leydigii*.  
 1. Myxosporidia from gall bladder of *Torpedo torpedo* (after Leydig.  $\times \frac{3}{2}$ ).  
 1a. Without pansporoblasts.  
 1b. With pansporoblasts and spores.  
 1c. With pansporoblast and sporoblast.  
 2a-b. Myxosporidia from gall bladder of *Scylliorhinus canicula* (after Leydig.  $\times \frac{3}{2}$ ).  
 2a<sub>1</sub>, a<sub>2</sub>. Myxosporidia without pansporoblasts.  
 2b. Myxosporidium with 12 pansporoblasts, each containing 1 spore.  
 3. Myxosporidium. This figure appears to be generalized from figures a<sub>1</sub>, a<sub>2</sub>, of the preceding (after Leuekart.  $\times 1$ ).  
 Fig. 4. *Chloromyxum fluviatile* (after Thélohan.  $\times \frac{3}{2}$ ). Vertical view showing the capsules in 2 lateral pairs, the nonvacuolate sporoplasm, the vertical position of the ridge, and the minute spines on the shell.  
 Figs. 5, 6. *Chloromyxum mucronatum*.  
 5a, b. From urinary bladder of *Lota lota* (after Lieberkühn.  $\times \frac{3}{2}$ ).  
 5a. Longitudinal view of spore, showing the 4 capsules.  
 5b. Vertical view showing the mucronate anterior extremity, capsules, and sporoplasm.  
 6. From *Lota lota* (after Balbiani.  $\times \frac{3}{2}$ ).  
 6a. Vertical view showing capsules, pericorncal nuclei, and vertical position of the ridge.  
 6b. The same; also beginning of valve separation.  
 6c. The same; also corkscrew extrusion of filaments.

## PLATE 40.

- Fig. 1 *a-c*. *Chloromyxum elegans* (original enlargement from plate 31, fig. 2.  $\times$  about 3). Three views of spores, showing outline, ridge, and capsules.
- Fig. 2 *a-b*. *Chloromyxum perlatum* (after Balbiani.  $\times \frac{3}{2}$ ). Vertical views of spores showing outline, capsules (*b* with filaments extruded), and vertical position of ridge.
- Fig. 3. *Chloromyxum* sp. 91. Vertical (?) view of spore from the ovary of *Lota lota* (after Bütschli.  $\times \frac{3}{2}$ ).  $\times$  about 900.
- Figs. 4-7. *Chloromyxum dujardini*.
4. From *Leuciscus rutilus* (after Müller.  $\times 1$ ).
  - 4*b*. Vertical views.
  - 4*c*. Transverse views.
  5. Myxosporidium from branchiæ of *Leuciscus erythrophthalmus* (after Dujardin.  $\times \frac{3}{2}$ ).  $\times 12$ .
  6. Spore showing outline and capsules; from *L. erythrophthalmus* (after Dujardin.  $\times 1$ ).  $\times 800$ .
  7. Free amœboid myxosporidium from a branchial lamella of *Leuciscus erythrophthalmus* (after Bütschli  $\times \frac{3}{2}$ ).  $\times$  about 30.
- Fig. 8. *Chloromyxum ohlmacheri* (after Ohlmacher, Leitz obj. 3, oc. 4.  $\times 1$ ). From photomicrograph of section of kidney; showing at *a*, and elsewhere, myxosporidian masses in the tubules; at *b* extravasated blood corpuscles; at *c* a large blood vessel filled with blood corpuscles. Fuchsin and iodine green.

## PLATE 41.

- Figs. 1-3. *Chloromyxum ohlmacheri*.
1. Spores (after Ohlmacher. Leitz pantachromatic oil imm. 2 mm., oc. 4.  $\times 1$ ).
  - 1*a*. Vertical view of spore, showing capsules with extruded filaments. Camera lucida; Babes's anilin water safranin.
  - 1*b*. Vertical view showing capsules, spiral-coil structure of shell, and vertical position of ridge.
  - 1*c*. Striæ are seen "running nearly meridionally"; at one "side" of spore a capsule "appears in the act of escaping through a rent" in the shell.
  - 1*d*. Fragment of shell in which the striæ appear to correspond to ridges encircling the shell.
  2. Kidney tubule, inclosing 3 spores, showing capsules and sporoplasm, the latter structure being represented in 1 spore as divided into 2 lateral halves. (An error; see p. 270.) Pfützner's alcoholic safranin (after Ohlmacher; camera lucida; Leitz pantachromatic 3 mm., oc. 4.  $\times 1$ ).
  3. Diagrammatic figure of spore; *a*, shell; *b*, sporoplasm; *c*, capsule; *d*, posterior extremity of ridge and spore; *e*, ridge; *f*, anterior extremity of ridge and spore; *g*, filaments, much shortened; *a*, *b*, *c*, are on the left side of spore; *e* on the right. (After Whinery.  $\times 1$ ).
- Fig. 4. *Ceratomyxa sphaerulosa*. Spore showing hollow-cone valves, vertical ridge, and valve-junction plane, capsules, and (*spo.*) the unilateral sporoplasm, and (*x*) pale corpuscles of indeterminate nature (after Thélohan.  $\times \frac{3}{2}$ ).

## PLATE 42.

- Figs. 1-10. *Cystodiscus immersus* (after Lutz.  $\times 1$ ).
1. Gall bladder of *Bufo aqua* with myxosporidium disks shining through.  $\times 1$ .
  2. Portion of medium-sized specimen with large number of spores.  $\times$  about 70.
  3. The same; the ruptured ectoplasm permitting the exit of the contents in the form of vesicles.  $\times$  about 70.
  4. Ripe spore-pairs.
  5. Vertical (?) view of mature spores, showing ridge.
  6. Longitudinal (?) view of same.
  7. Spore with extruded filaments, showing the striæ of the shell.
  8. Spore with valves separated.
  9. Developmental condition of spore.
  10. Mature spore; contents made plain by carmine; containing micrococcoid granules.  $\times$  about 600.
- Figs. 11-13. *Cystodiscus ?? diploxyis* (after Balbiani).
- 11, 12. Spherical cysts in process of spore formation ( $\times 1$ ).  $\times 85$ .
  13. Spores from the cysts ( $\times \frac{3}{2}$ ).  $\times$  about 1500.
  - 13*a*. Vertical view.
  - 13*b, c*. Transverse views.

## PLATE 43.

Figs. 1-5. *Myxidium lieberkühni*.

1. Myxosporidia (after Lieberkühn.  $\times 1$ ).
- 1a. Showing the granule-free, pronged end by which attachment is effected, and a pansporoblast containing 2 spores.  $\times 330$ .
- 1b. Myxosporidium which has mostly broken up into pansporoblasts.  $\times 900$ .
2. Specimen covered with transverse wrinkle-like elevations; at one end some pseudopodia (after Bütschli.  $\times \frac{2}{3}$ ).  $\times 160$ .
3. Three successive stages in the development of clear ectoplasmic pseudopodia at one end of a large myxosporidium (after Bütschli.  $\times 1$ ).
4. Small myxosporidium attached to a nucleated bladder cell (after Bütschli.  $\times 1$ ).
5. Strongly amœboid-branched specimen (after Bütschli.  $\times \frac{2}{3}$ ).  $\times$  about 90.

## PLATE 44.

Figs. 1-5. *Myxidium lieberkühni* (after Bütschli.  $\times 1$ ).

- 1a, b. Large forking myxosporidia; a, with fine hair-like ectoplasmic processes.
2. Large myxosporidium, showing interlaminae between ectoplasm and endoplasm.
3. Portion of border of myxosporidium showing the peculiar canaliculate structure mentioned on p. 285.
4. Part of border of large myxosporidium with branched horn-like ectoplasmic processes.
- 5a-d. Four yellowish fat globules, inclosing hæmatoidin crystals.

## PLATE 45.

Figs. 1-3. *Myxidium lieberkühni* (after Pfeiffer.  $\times 1$ ).

- 1a. Smallest form.
- 1b. Small form with fat globules, hæmatoidin crystals, with only 1 pair of ripened spores; ectoplasm evident.
- 1c. Motile myxosporidium with very strong soap-bubble-like ectoplasm; in its interior a well-preserved red blood corpuscle, with fat globules and hæmatoidin inclusions.
- 1d. Specimen with amœboid pseudopodia.
- 1e, f. Large forms with scattered spores.
- 1g. Carmine staining after removal of fat by chloroform; the whole endoplasm riddled with nuclei. As yet without spores.
- 1h. Isolated spore.  $\times 1200$ .
- 2, I. Superficial epithelial layer; 6 healthy epithelial cells with nuclei, and 2 separate strongly hypertrophied cells in which, very soon after infection, the nucleus is destroyed.
- 2, IIa. Myxosporidium fallen out of epithelial cell. Still without ectoplasm.
- b. Young form, free in urine with peculiar pseudopodioid motile ectoplasmic processes extruded and retracted on a slightly warm stage and many fat globules in the endoplasm.
- 2, IIIa. Pansporoblast formation; a, small myxosporidium with bristle processes.
- b. Sexanucleate pansporoblasts, which later form 2 trinucleate sporoblasts; in each sporoblast 2 nuclei form the capsules, the third the sporoplasm (*vide* Pfeiffer).
3. Transverse section of urinary bladder of pike, alcohol-hardened, celloidin-embedded, hæmatoxylin-stained.
- 3a. Showing, from right to left, the external muscle layer, the internal muscle layer cut transversely, the submucosa, the epithelium with infection in the superficial layers, and free brown-colored myxosporidia containing hæmatoidin and sporoblasts.  $\times 80$ .
- 3b. Portion of a. To the right the monstrosously appearing myxosporidia and sporoblasts.  $\times 400$ .
- 3c. Natural size of the bladder section.

## PLATE 46.

Figs. 1-3. *Myxidium lieberkühni*.

1. Epithelial infection of bladder from fresh and also from hæmatoxylin-stained material (after Pfeiffer.  $\times 1$ ).
- 1a. To the left healthy, to the right slightly hypertrophied epithelia which have lost their nuclei. At the right border, monstrously enlarged epithelia, or rather myxosporidia, with fat and hæmatoidin contents; nucleus obscure. Below to the left an isolated epithelial cell with early infection, and the disrupted epithelial nucleus.
- 1b. Immigration of young myxosporidia into the red blood corpuscles of *Lucius lucius*. Nucleus, where preserved, dark. In the upper row the middle corpuscle shows a multiple infection. Lower row showing not spore formation, but fat globules, nuclei, and hæmatoidin crystals. In the lower right-hand figure the myxosporidium has left the blood corpuscle and developed its hyaline ectoplasm.
2. Myxosporidia (after Balbiani.  $\times 1$ ).
- 2a. Myxosporidium filled with fatty granules without pansporoblasts.
- 2b. Myxosporidium with well-developed spores.
- 2c, d. Very young myxosporidia.
3. Pansporoblast containing 2 mature spores (after Lieberkühn.  $\times \frac{3}{2}$ ).

## PLATE 47.

Figs. 1-5. *Myxidium lieberkühni*.

1. Spore formation (after Bütschli.  $\times 1$ ).
- 1a. Pansporoblast with nuclei.
- 1b. The pansporoblast has contracted its bulk somewhat, elongated to an oval, and oriented its nuclei preliminary to division.
- 1c. The sexanucleate pansporoblast has divided into 2 spherical trinucleate sporoblasts.
- 1d. The sporoblasts have elongated and oriented themselves and their nuclei.
- 1e, f. Showing the development of the capsules independently of the vanishing terminal nuclei. In the center of the spore, its nucleus (see p. 287).
2. Developed spore (after Lieberkühn.  $\times \frac{3}{2}$ ).  $\times 900$ .
3. Mature spore (after Bütschli.  $\times 1$ ). Showing outline, bilateral symmetry, capsules, sporoplasm, and nucleus (see p. 287).
4. The same (after Balbiani.  $\times 1$ ).
- 4a, b. Most common form of spores with 1 capsule in each wing; b, with filaments extruded.
- 4c. Rarer form of spore with 2 capsules in each wing.
5. Spore with filaments extruded (after Bütschli.  $\times 1$ ).

Fig. 6. *Myxidium* ? sp. 102. Showing spore with capsules separated (? in each wing.) (After Leydig.  $\times \frac{3}{2}$ ).



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This index is intended as a supplement to, and not as a substitute for, the table of contents, tables of distribution, and the tabular key (pp. 138-165), and as a rule subjects embraced in those tables are not indexed. For the species occurring on any host, in any organ, or at any place, see *Distribution*, below. The following are, however, here included: (a) All myxosporidian (doubtfully myxosporidian, etc.) generic and specific names, including all synonyms; (b) all generic and specific names of hosts which have (in the myxosporidian literature) undergone changes of synonymy; (c) such common names of hosts as are well established. Authors included in the Bibliography (pp. 123-129) are omitted; all others cited are indexed.

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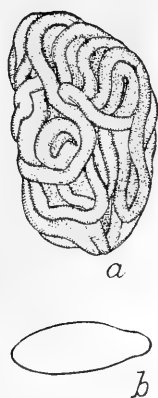


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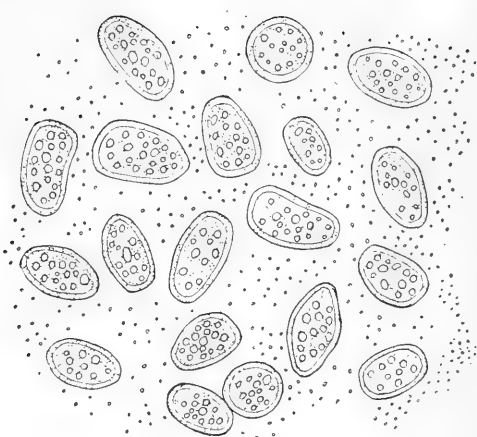


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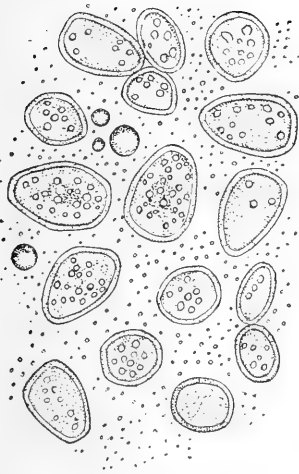


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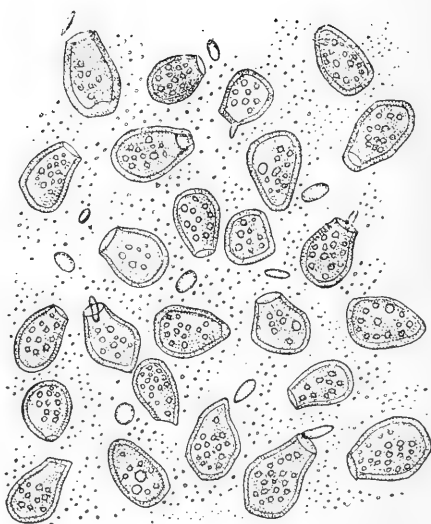
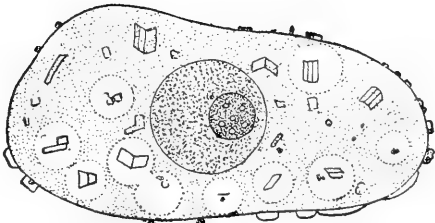
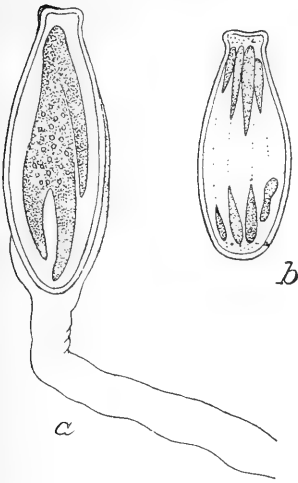


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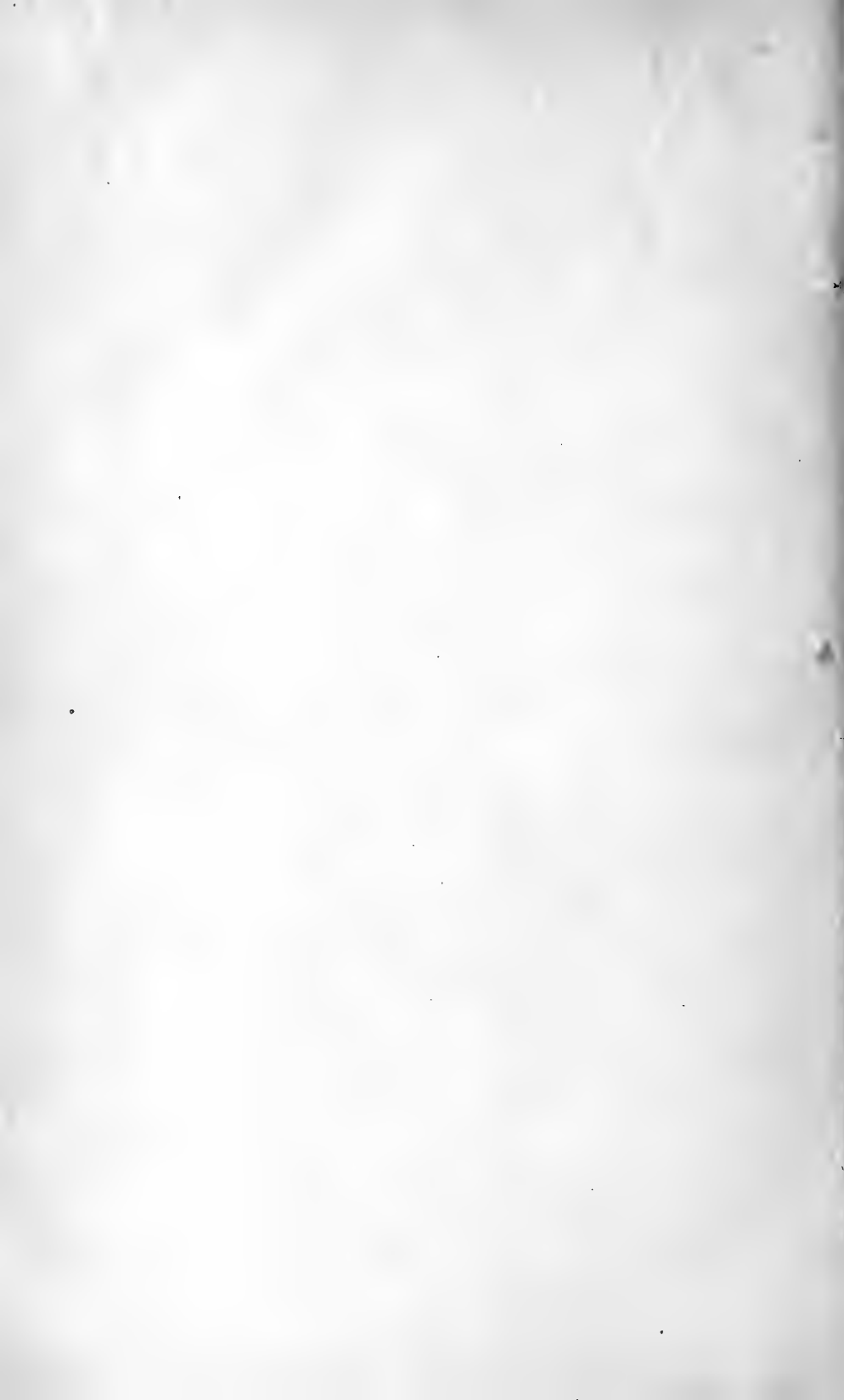


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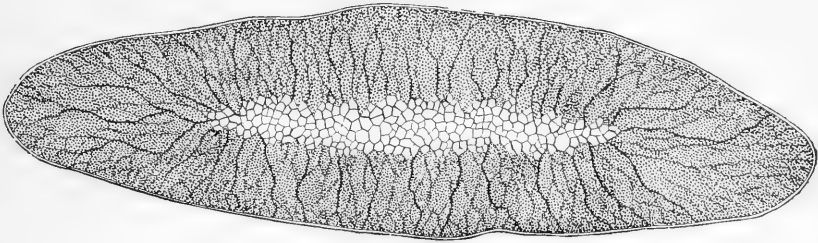


Fig. 1



Fig. 5



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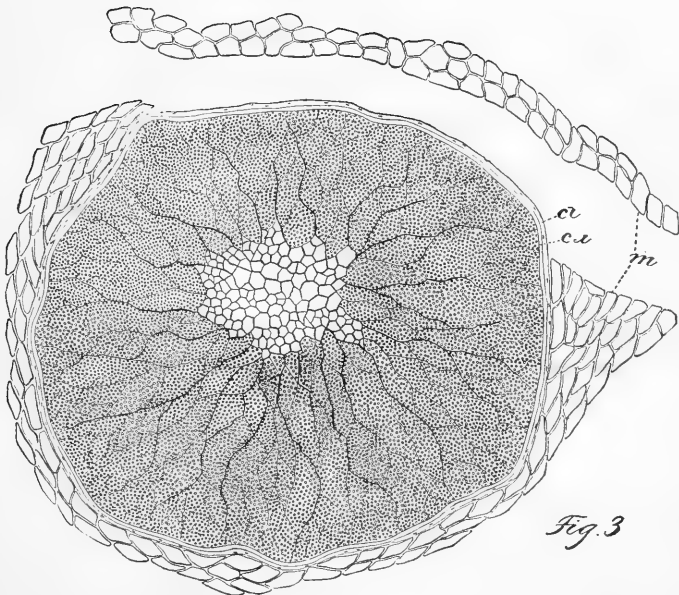
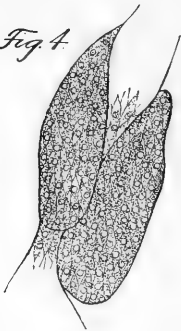


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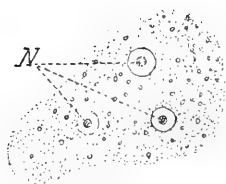
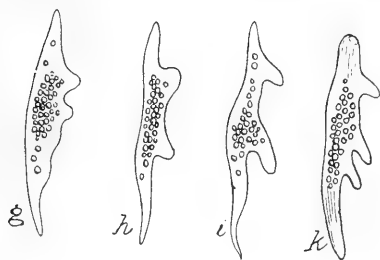


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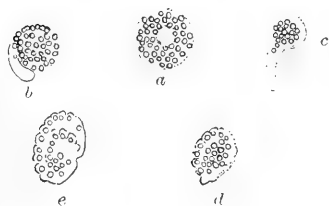
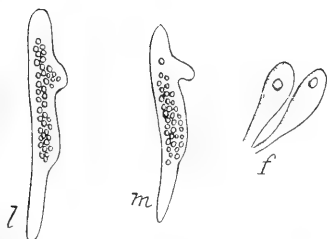


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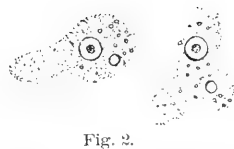


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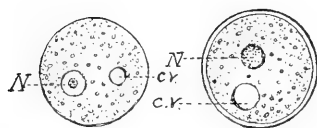


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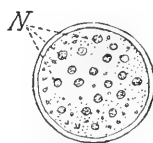


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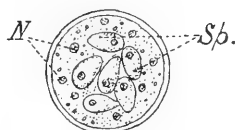


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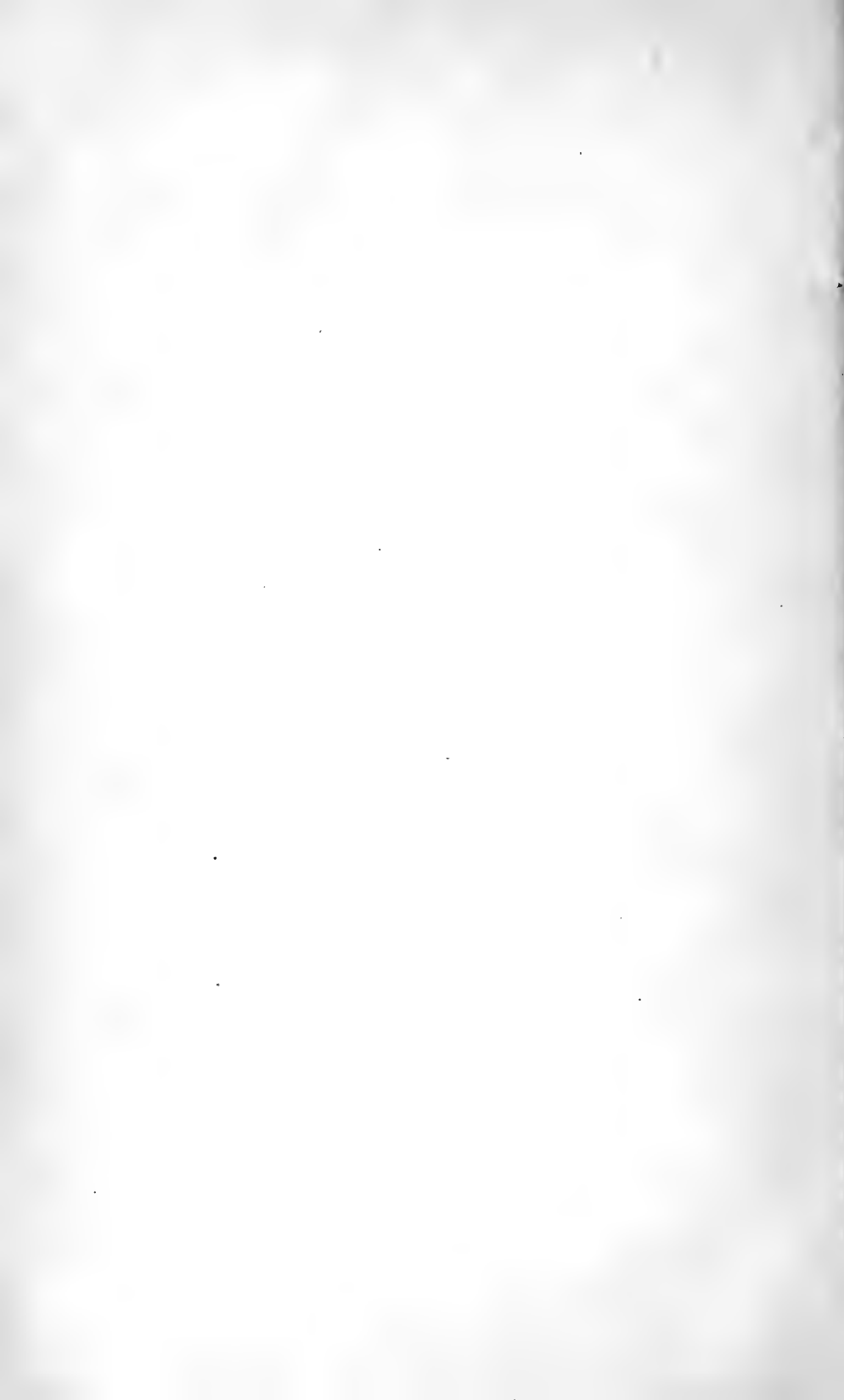




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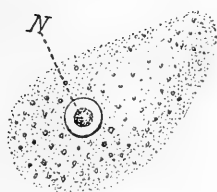


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Fig. 8.



Fig. 9.



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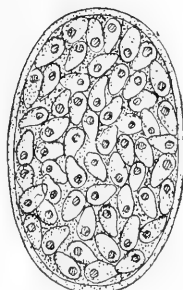


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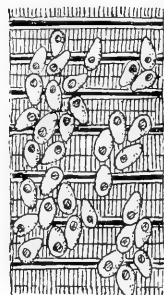


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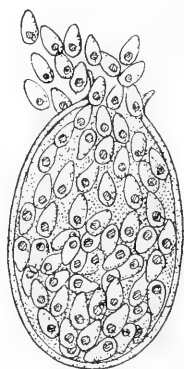


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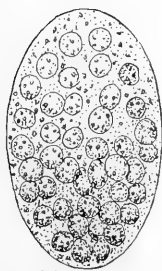


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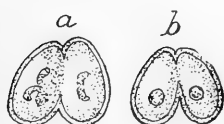


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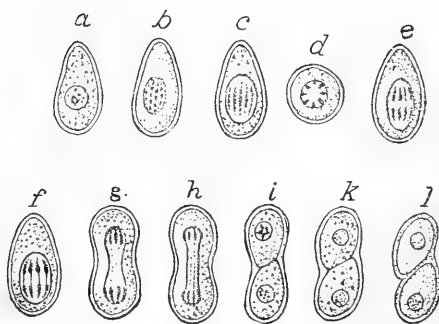


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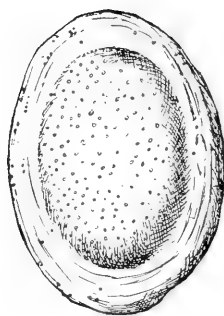


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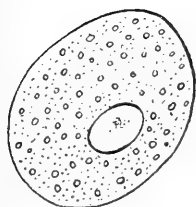


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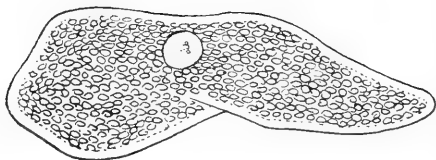


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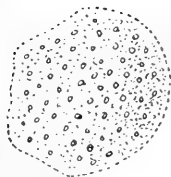


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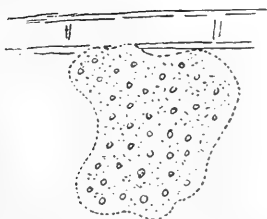


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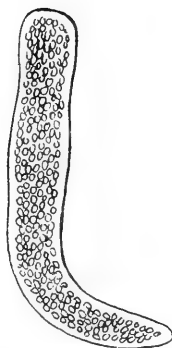


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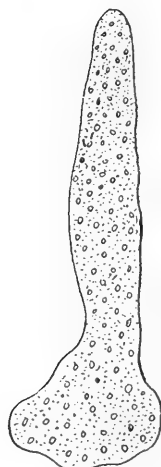


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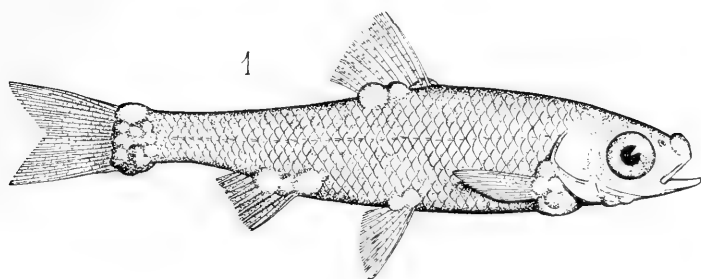


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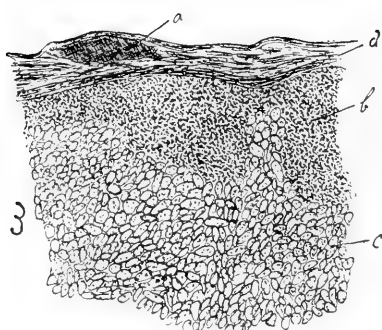


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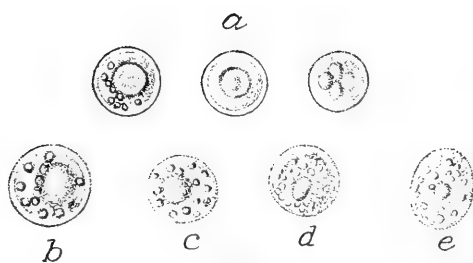
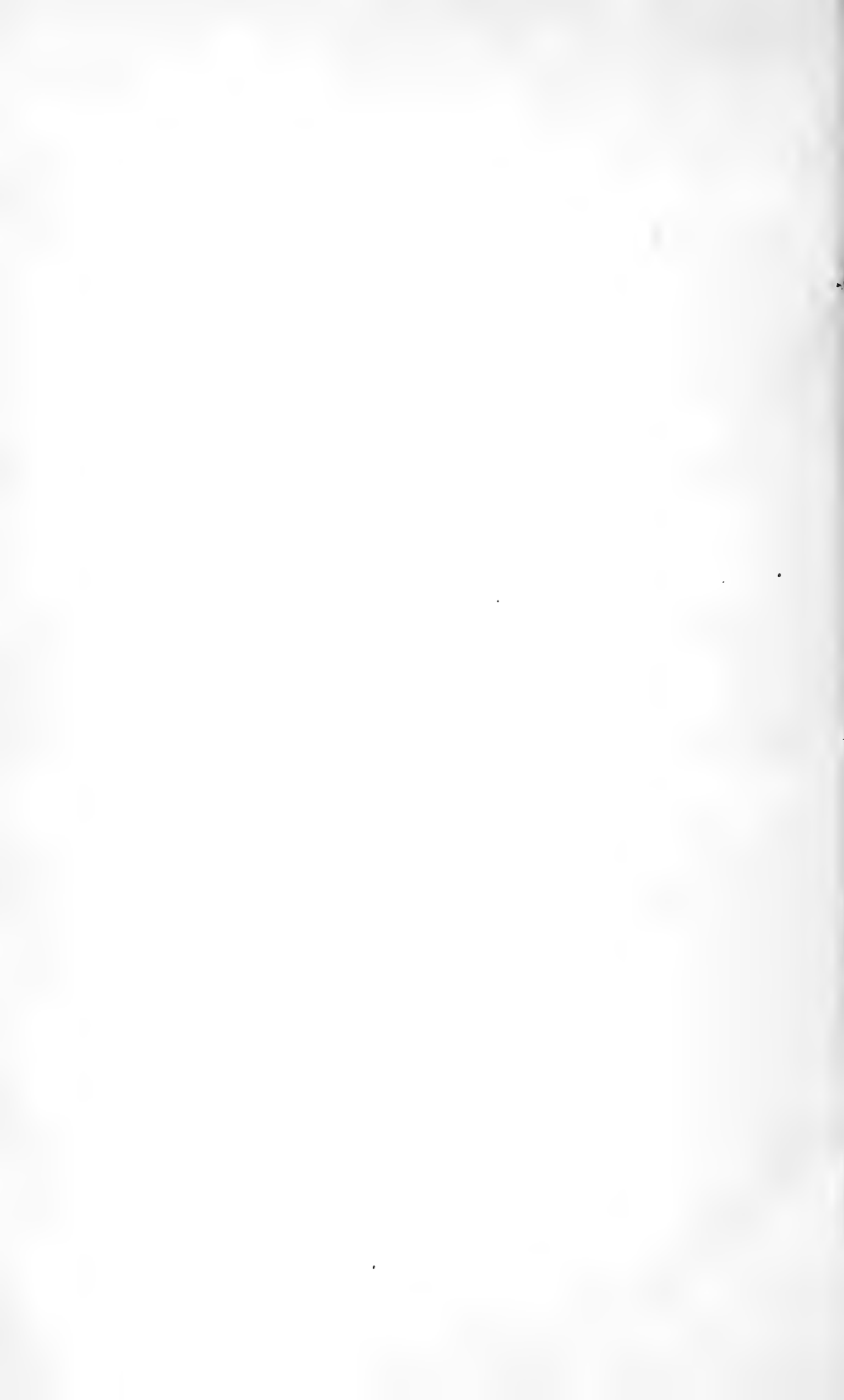


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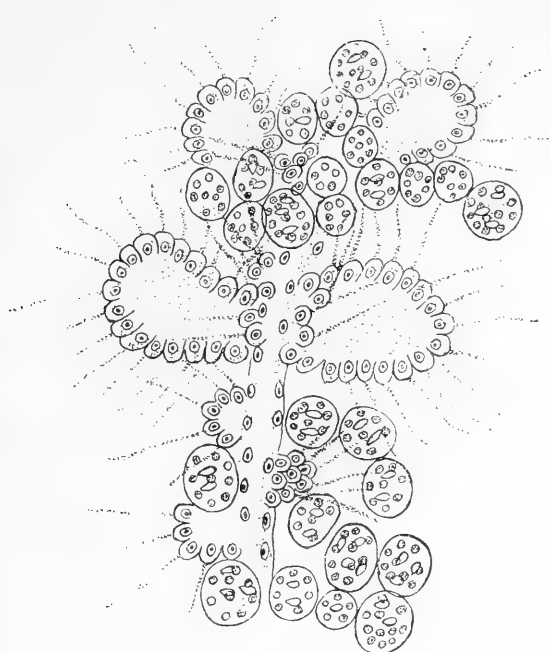


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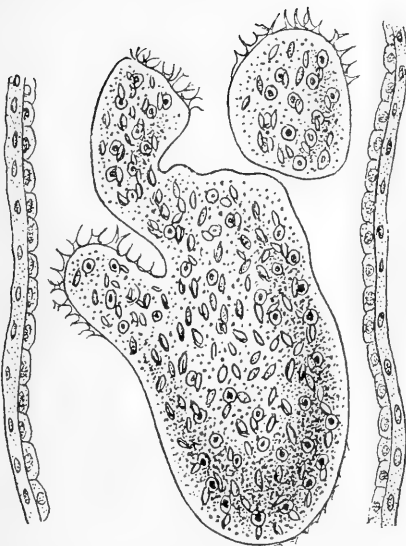


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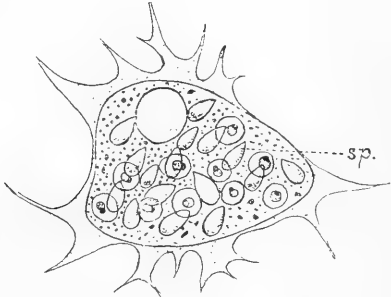


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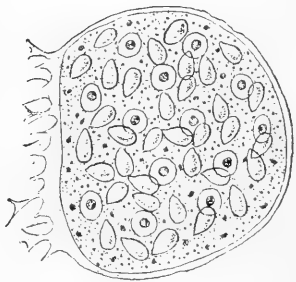
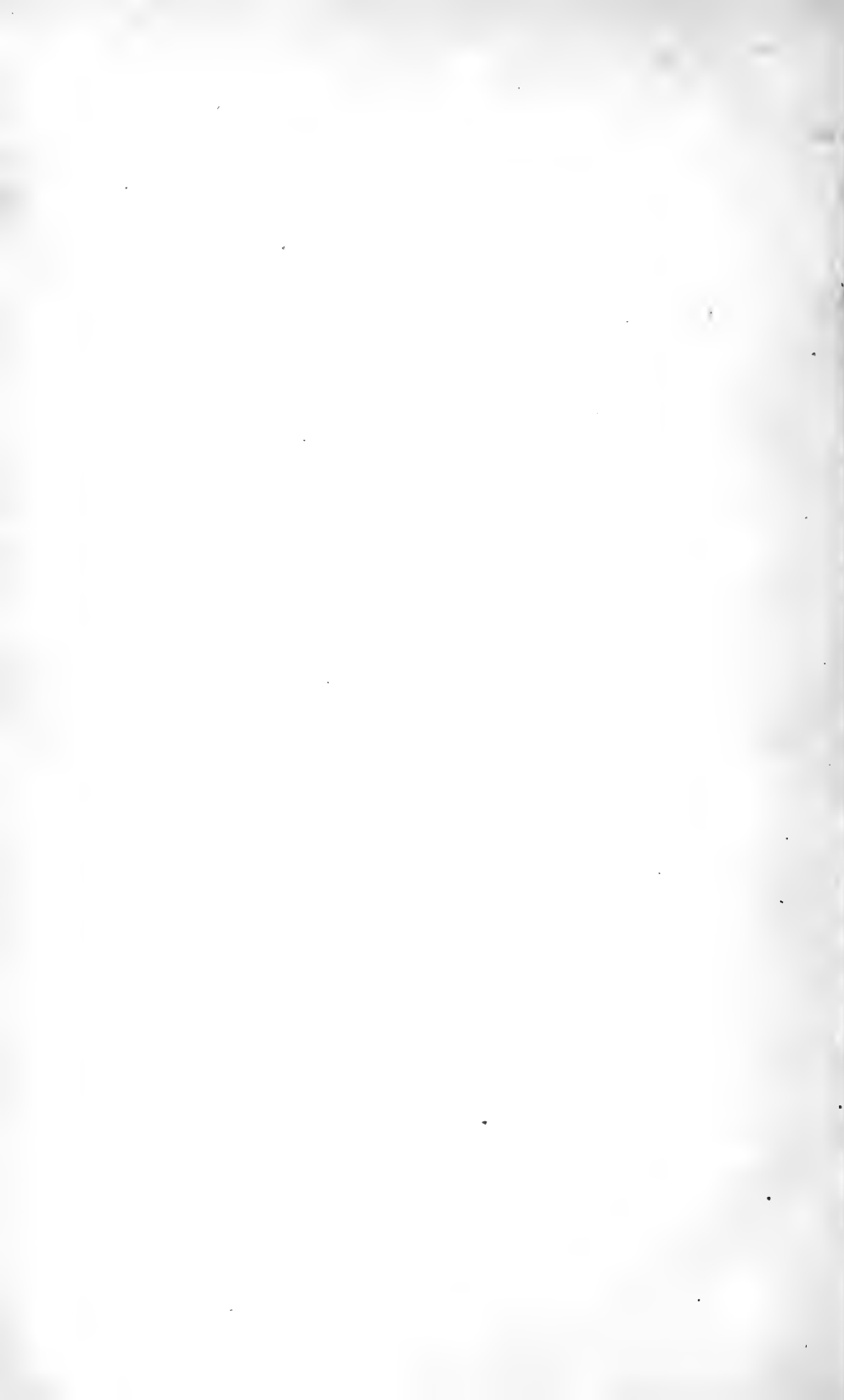


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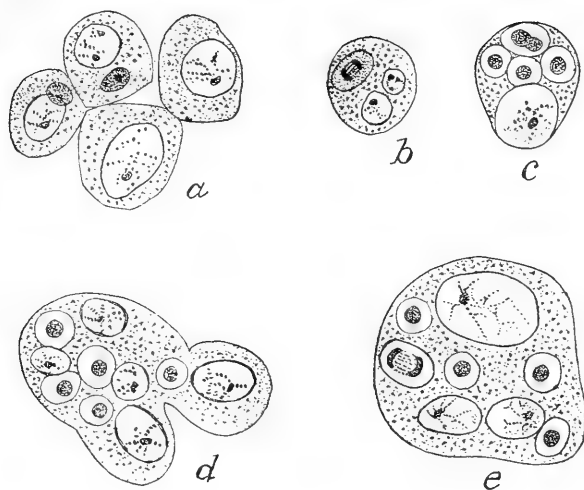


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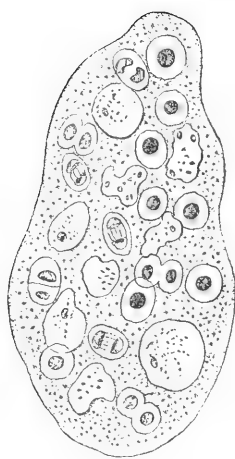


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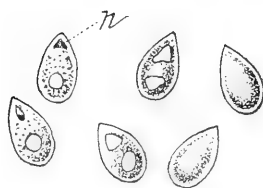


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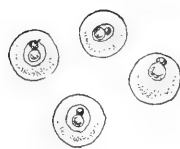


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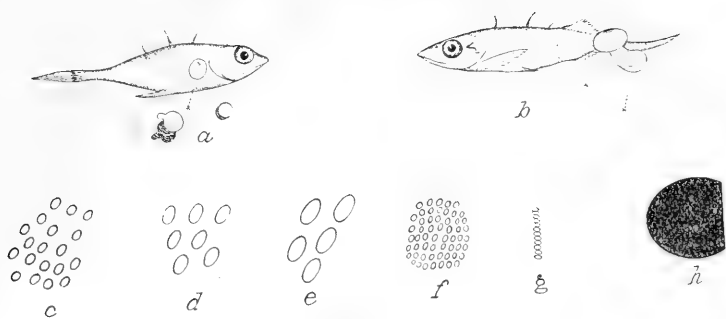


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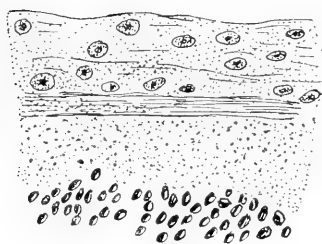


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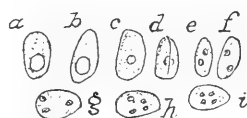


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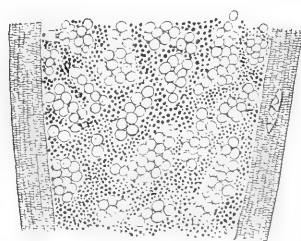


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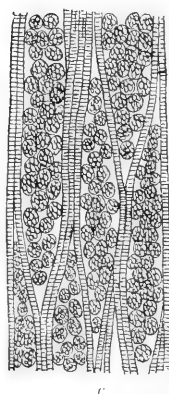
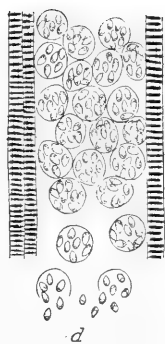


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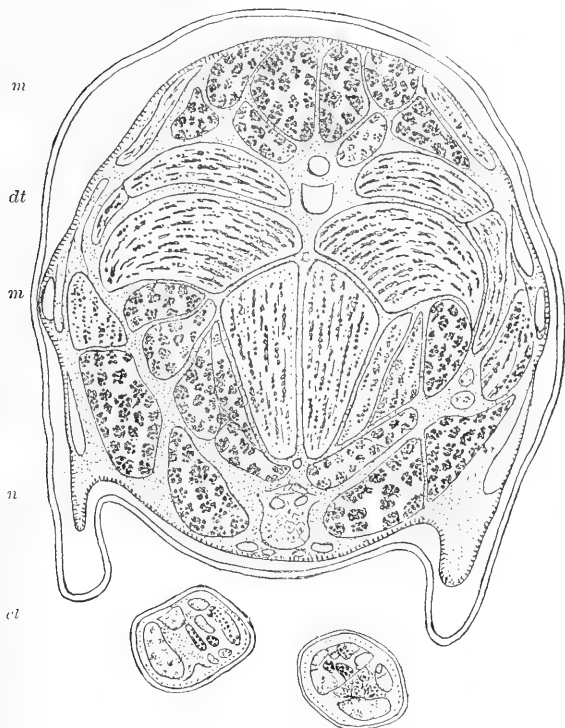


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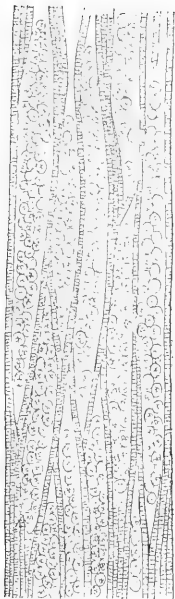


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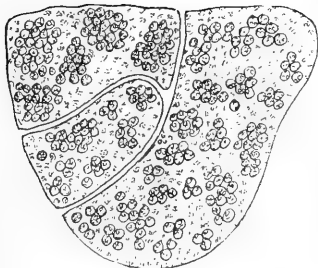


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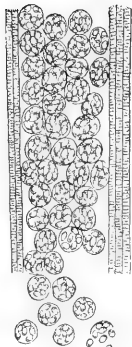


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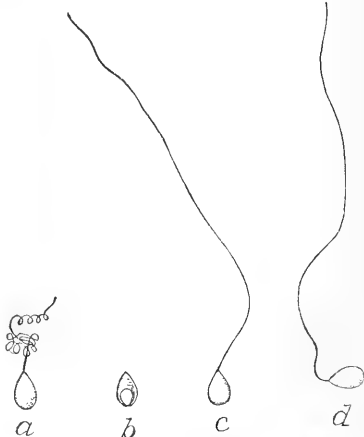


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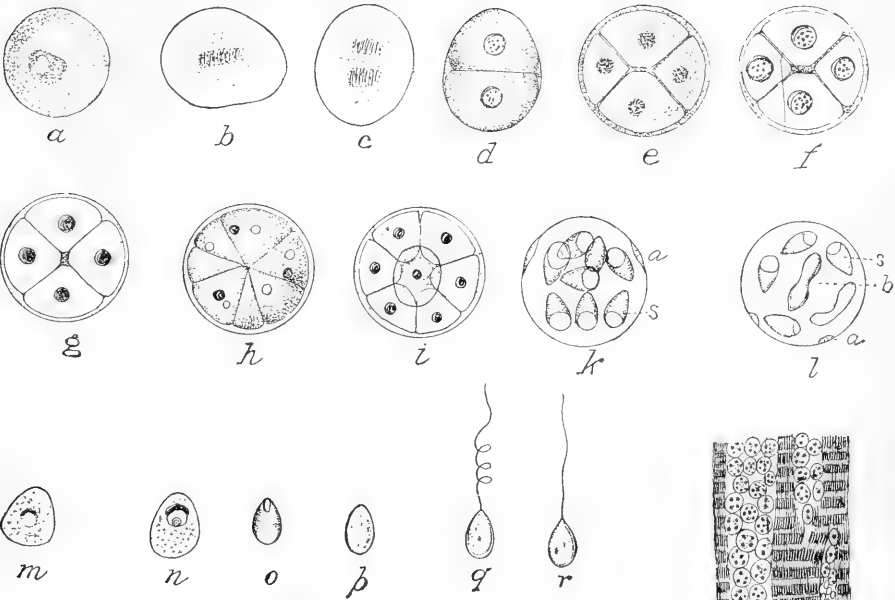


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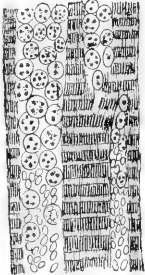


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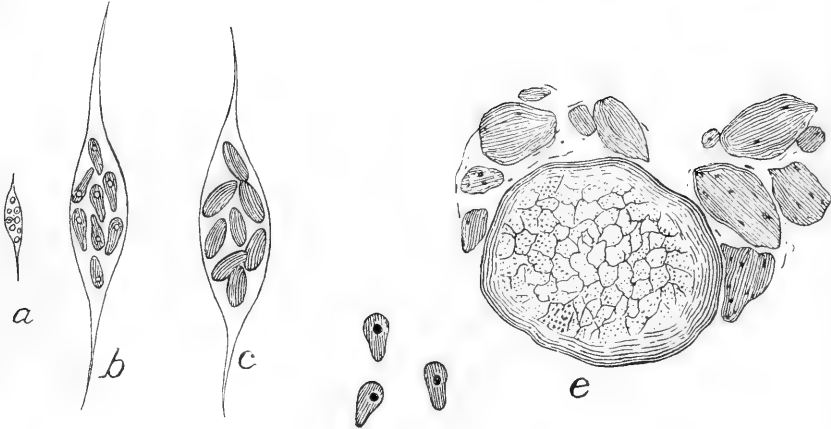


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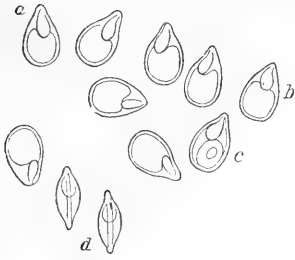


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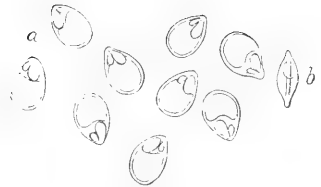


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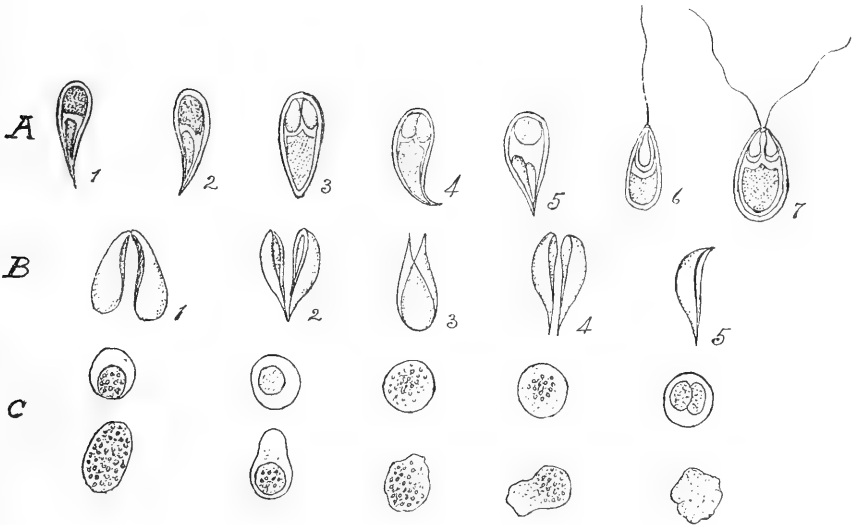


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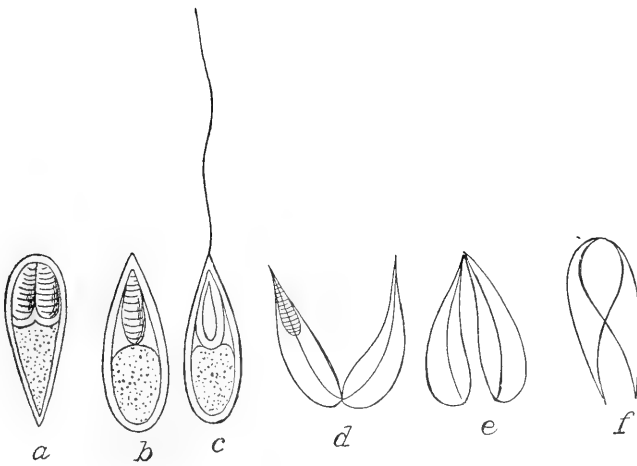


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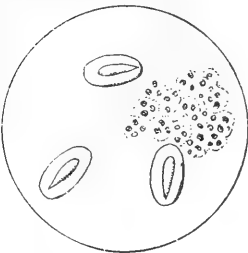


Fig. 1.



*a*



*b*



*c*



*d*



*e*

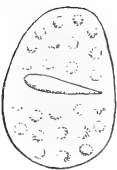


*f*



*g*

Fig. 4.



*a*



*b*



*c*

Fig. 2.



Fig. 3.

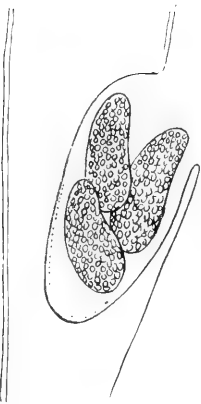


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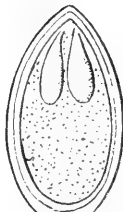


Fig. 6.



*a*



*b*

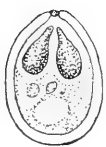
Fig. 7.



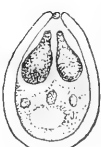
*a*



*b*



*c*



*d*

Fig. 8.





Fig. 1.



Fig. 3.

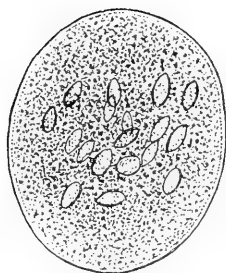


Fig. 4.

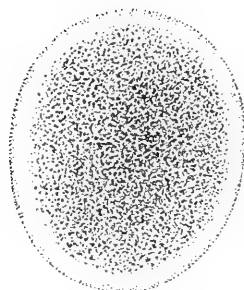


Fig. 2.



Fig. 6.

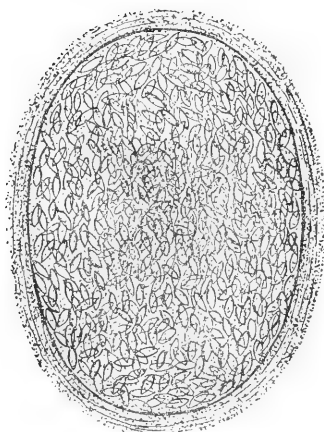


Fig. 5.

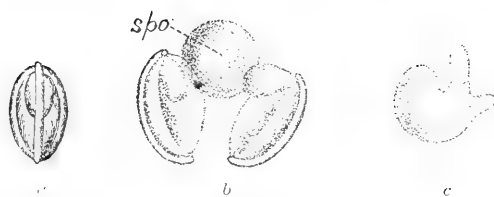


Fig. 7.



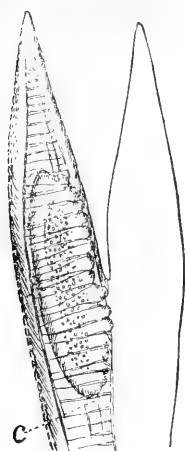


Fig. 1.



Fig. 2.

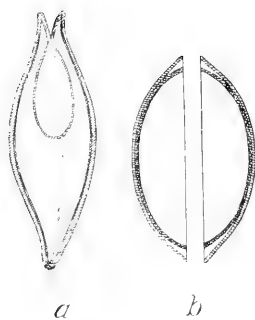
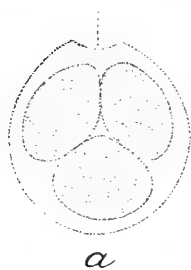


Fig. 3.



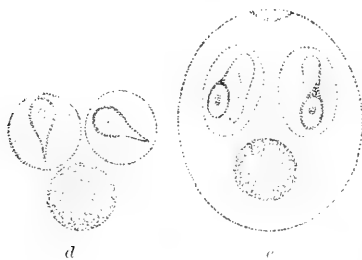
*a*



*b*



*c*



*d*

Fig. 4.



Fig. 5.

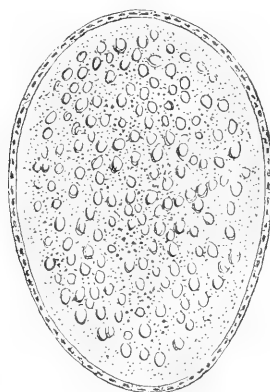


Fig. 6.



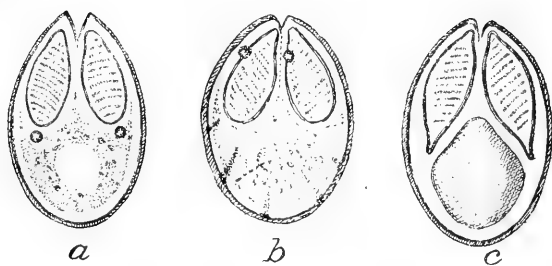


Fig. 1.

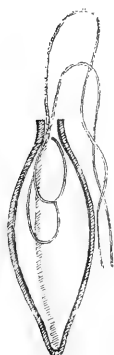


Fig. 2.



Fig. 3.

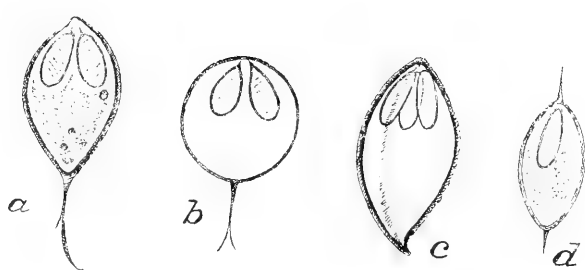


Fig. 4.



Fig. 7



Fig. 5.



Fig. 6.





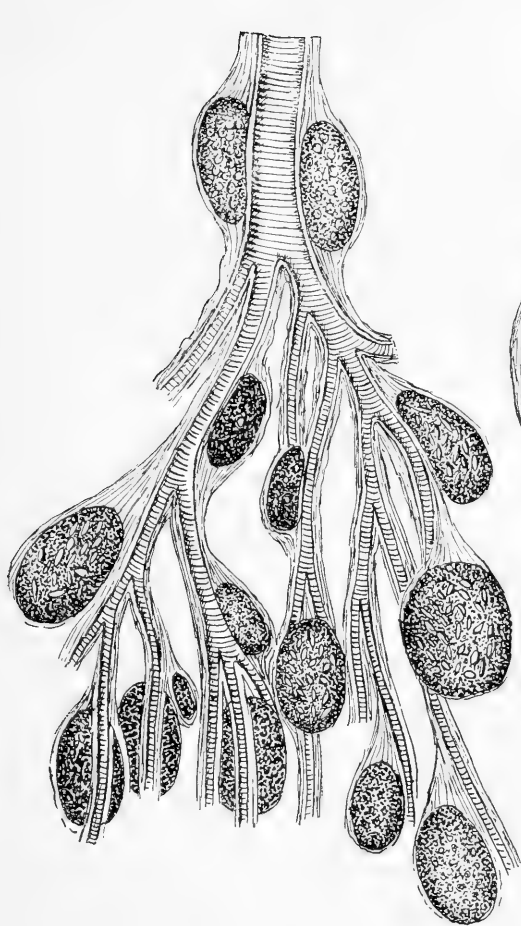


Fig. 1.

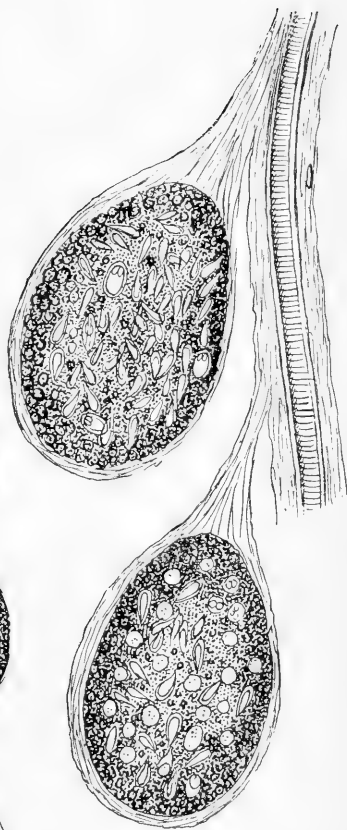


Fig. 2.





Fig. 1.



Fig. 2.



Fig. 3.

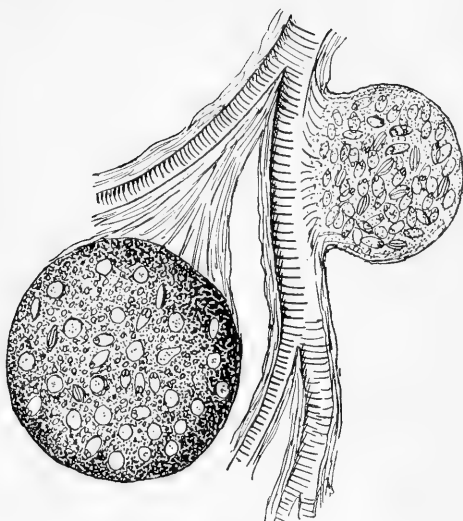


Fig. 4.

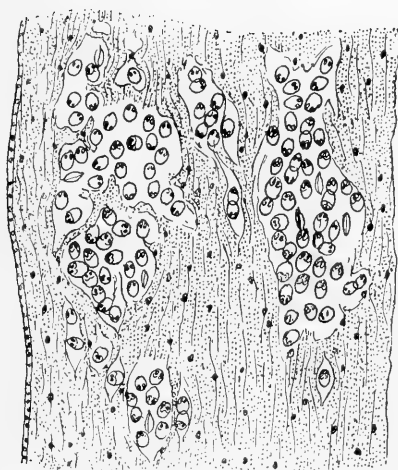


Fig. 5.

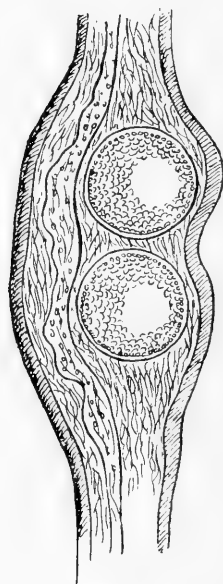


Fig. 7.

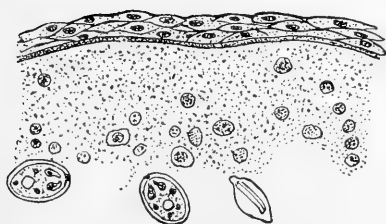


Fig. 6.



Fig. 8.



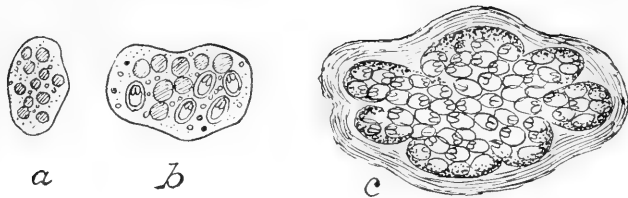


Fig. 1.

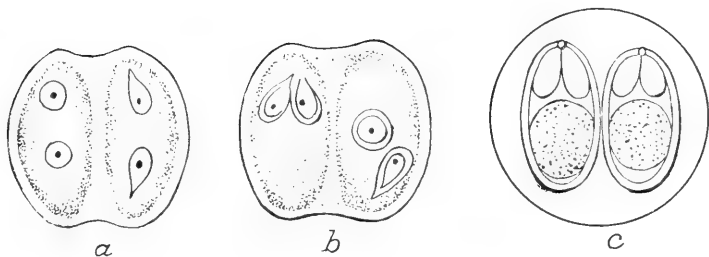


Fig. 2.

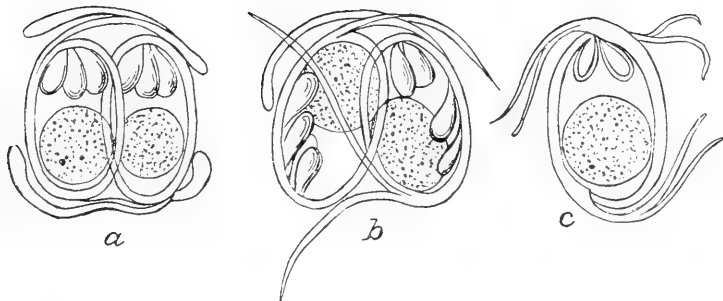


Fig. 3.

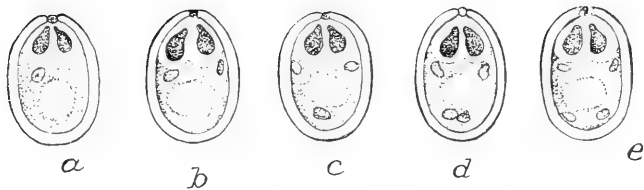
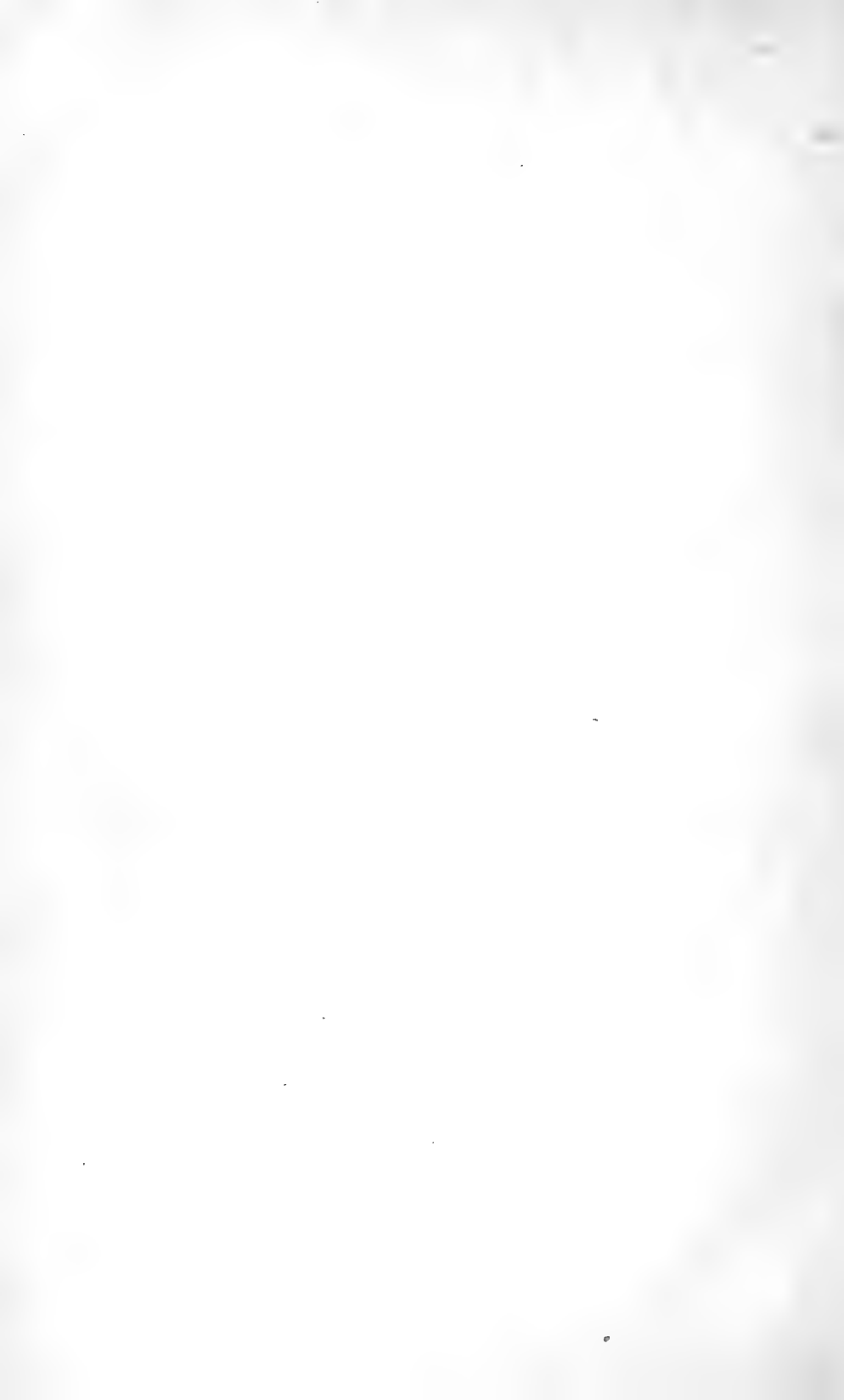


Fig. 4.



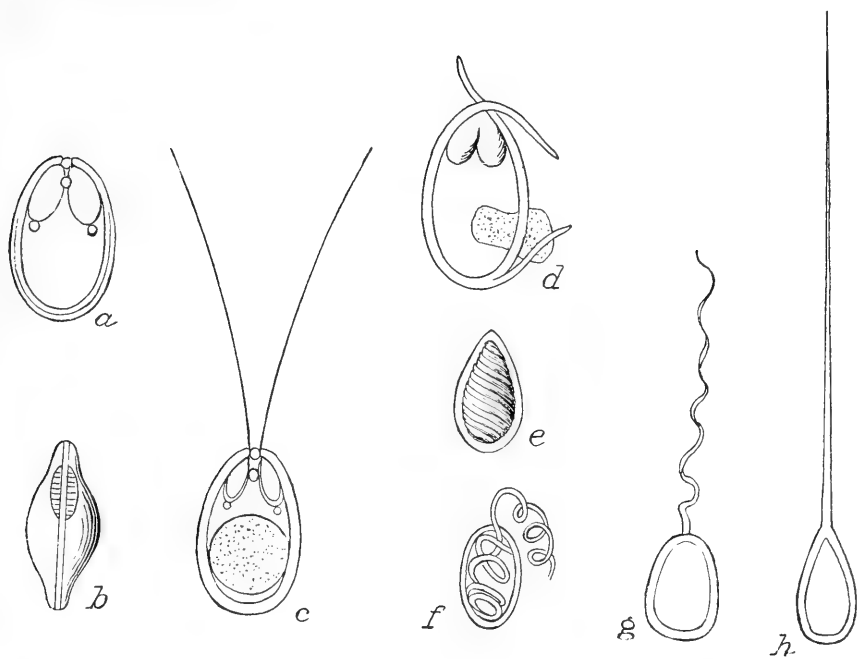


Fig. 1.

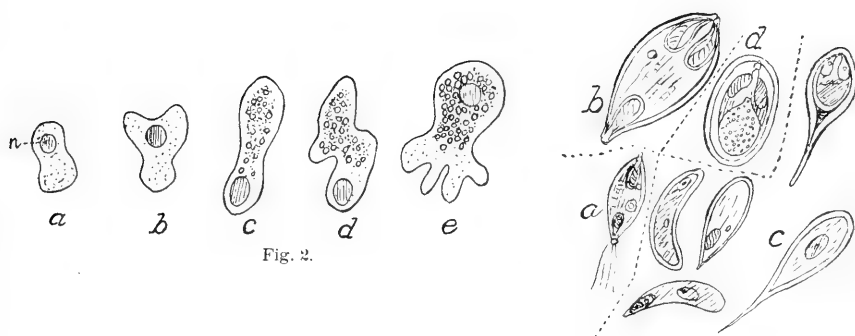


Fig. 2.

Fig. 3.

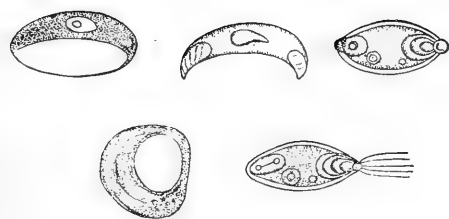


Fig. 4.



Fig. 5.







Fig. 1.

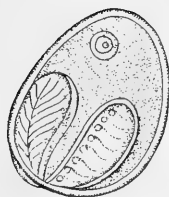


Fig. 2.

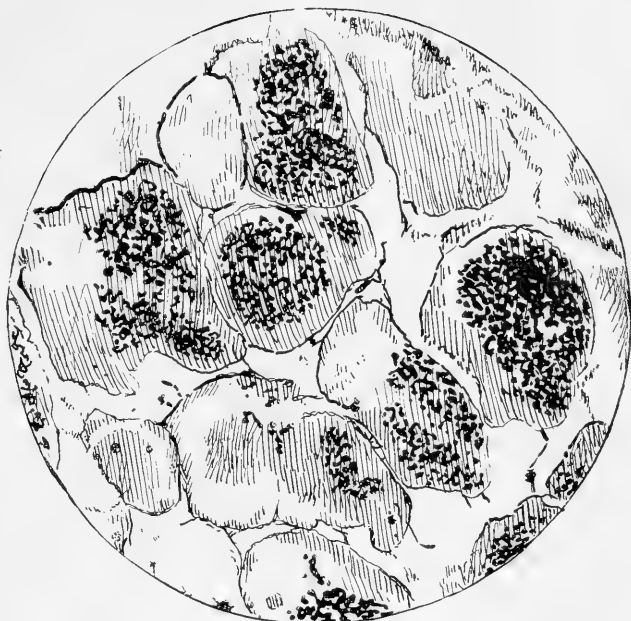


Fig. 5.

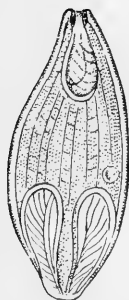


Fig. 3.



Fig. 4.

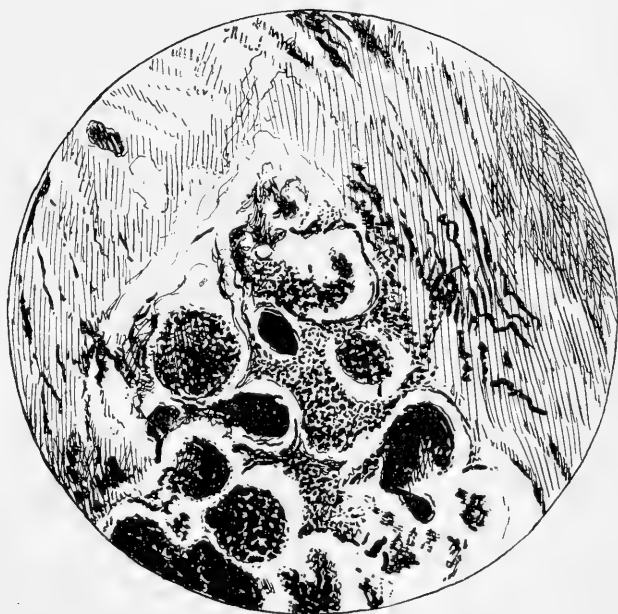


Fig. 6.



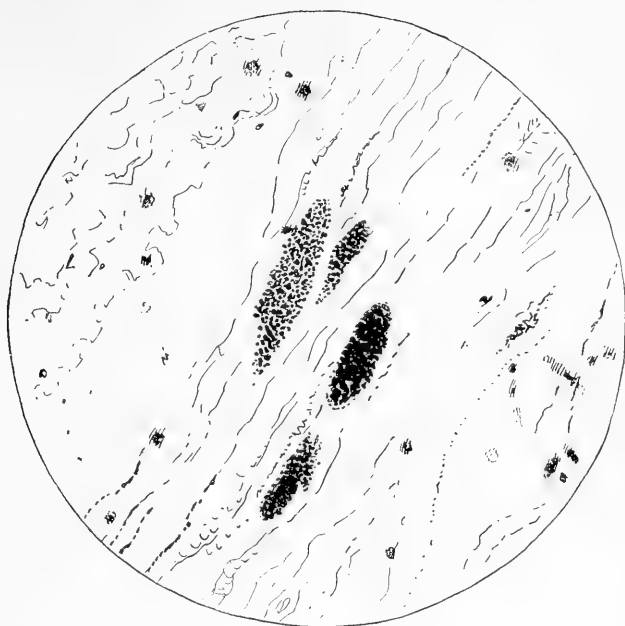


Fig. 1.

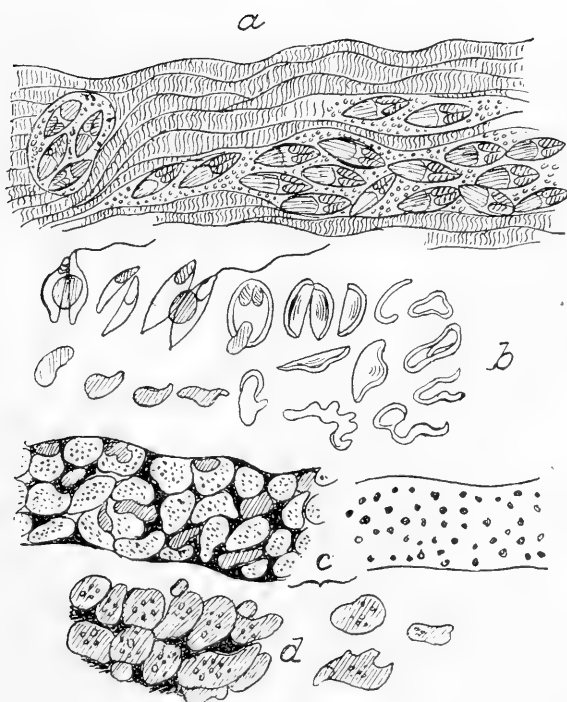


Fig. 2.



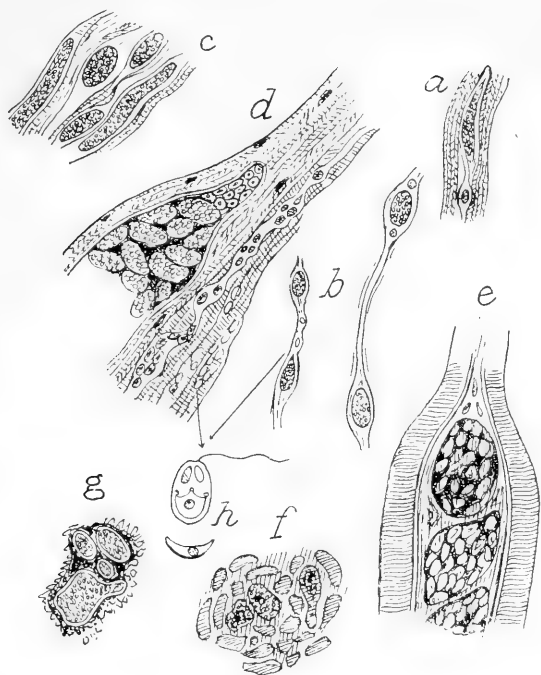


Fig. 1.

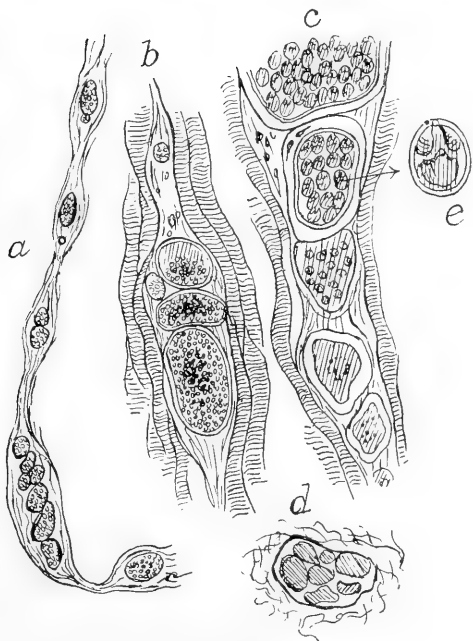


Fig. 2.



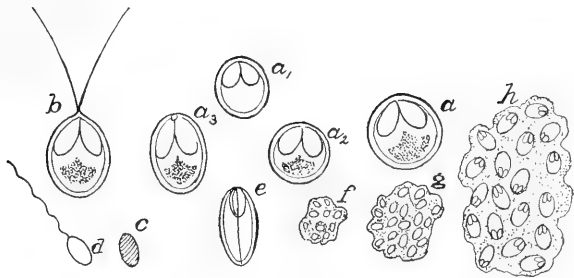


Fig. 1.

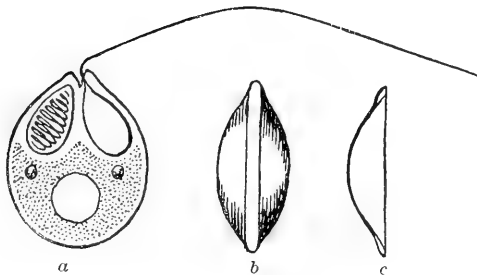


Fig. 2.



Fig. 3.



Fig. 4.

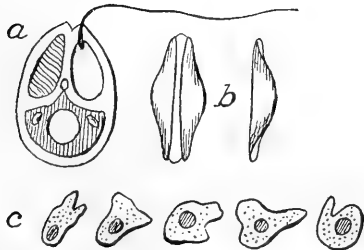


Fig. 5.



Fig. 6.







Fig. 1.



Fig. 2.

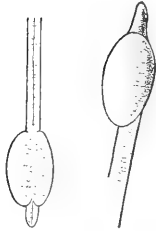


Fig. 4.



Fig. 3.



Fig. 5.

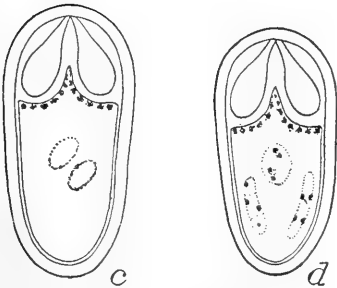
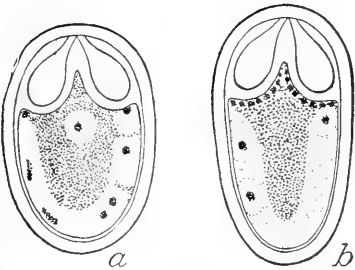


Fig. 6.

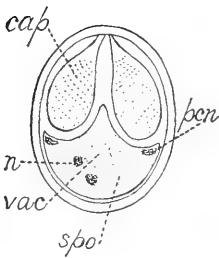


Fig. 7.

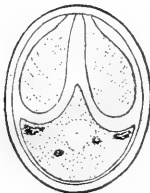
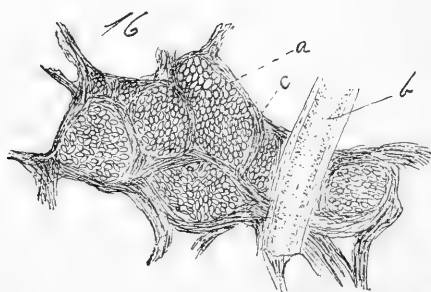
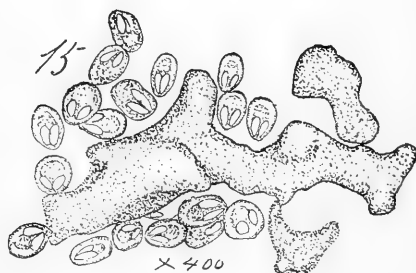
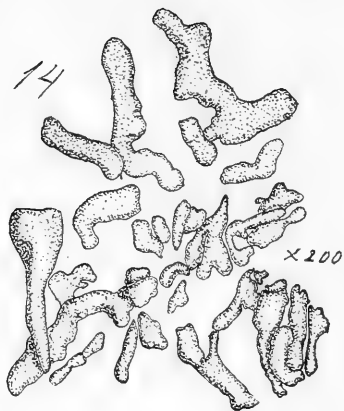
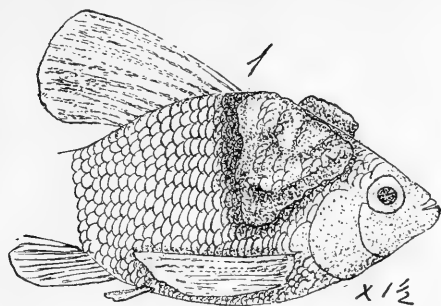


Fig. 8.







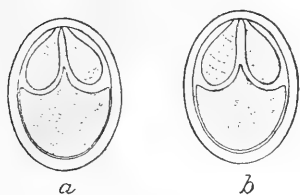


Fig. 4.

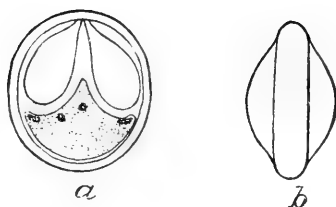


Fig. 1.

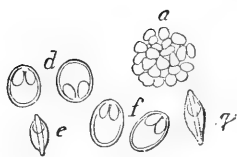


Fig. 5.



Fig. 2.

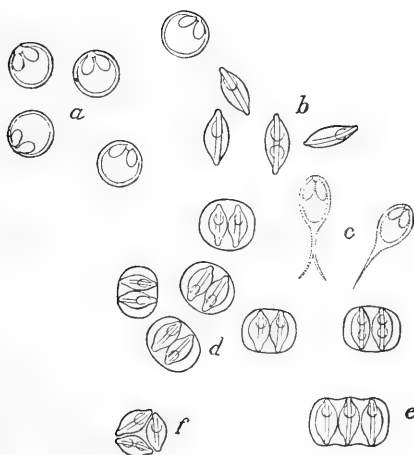


Fig. 3.

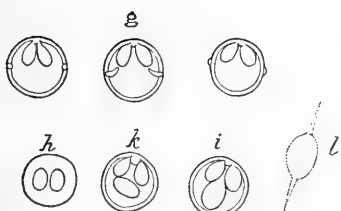


Fig. 6.

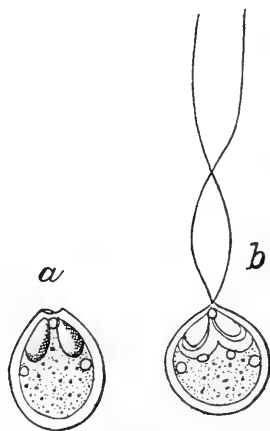


Fig. 7.



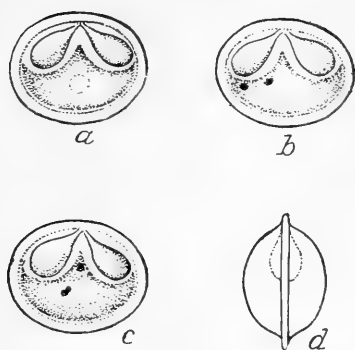


Fig. 1.

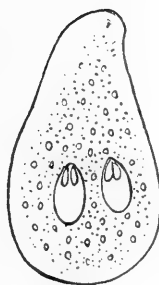


Fig. 2.



Fig. 3.



Fig. 4.

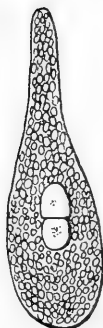


Fig. 5.

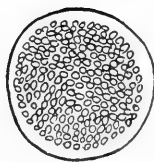


Fig. 6.

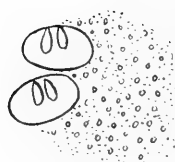


Fig. 7.

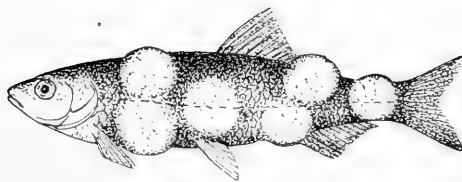


Fig. 8.





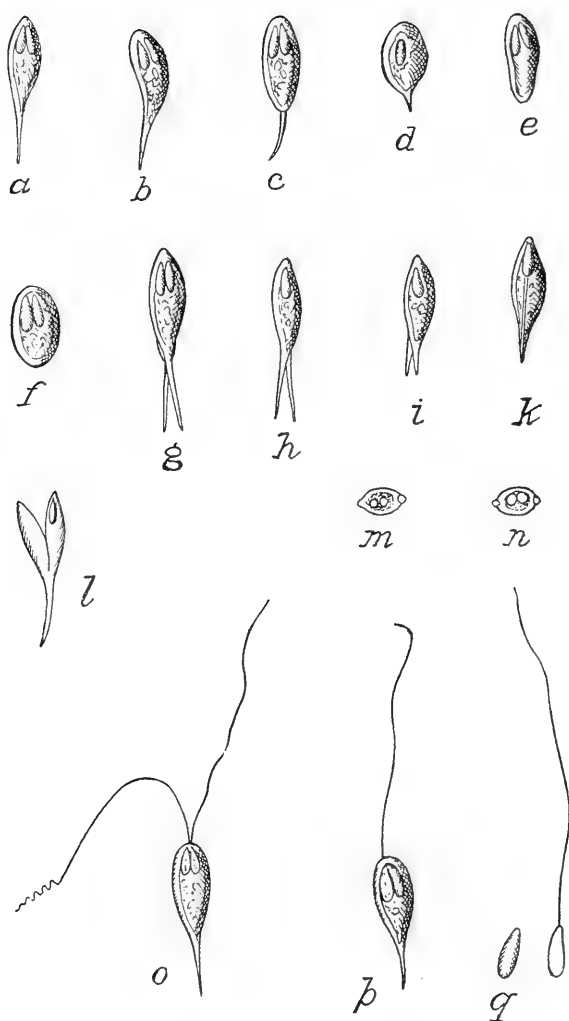


Fig. 1.



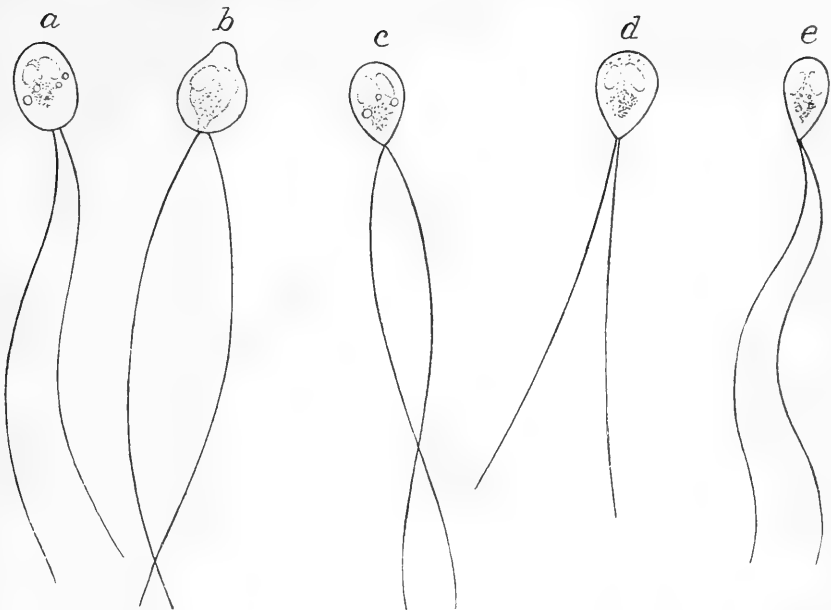


Fig. 1.

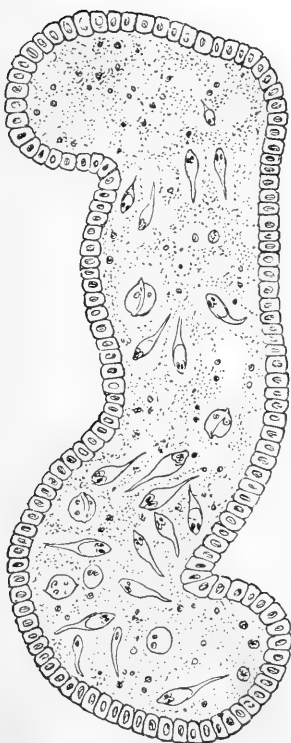


Fig. 2.

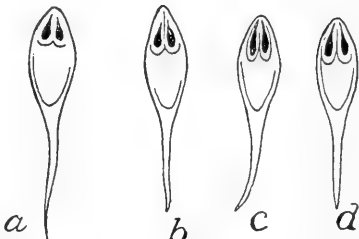


Fig. 3.



Fig. 4.



Fig. 5.



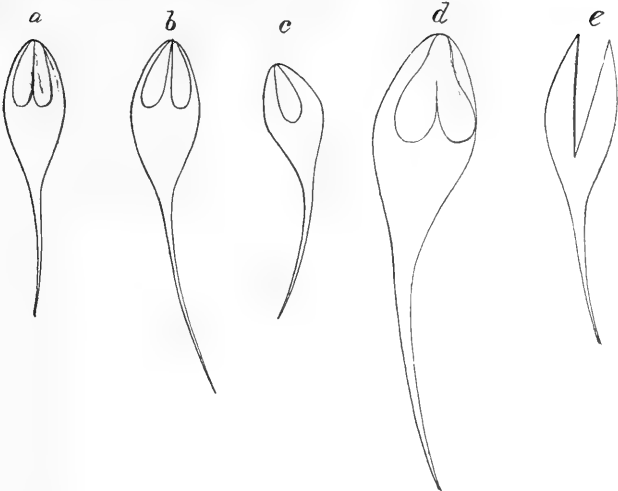


Fig. 1.



Fig. 2.

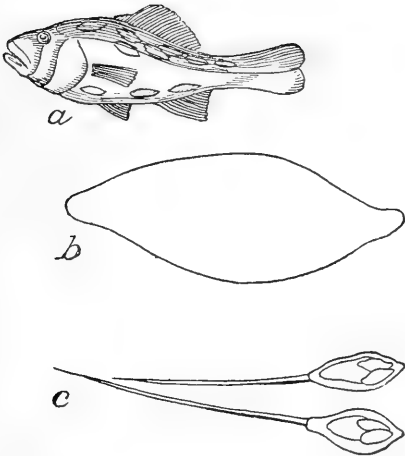


Fig. 3.

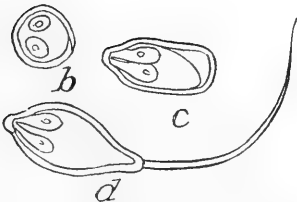


Fig. 4.



Fig. 5.





Fig. 1.

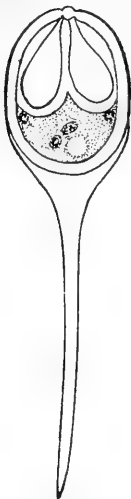


Fig. 2.

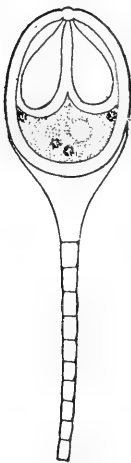


Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.



*a*



*b*

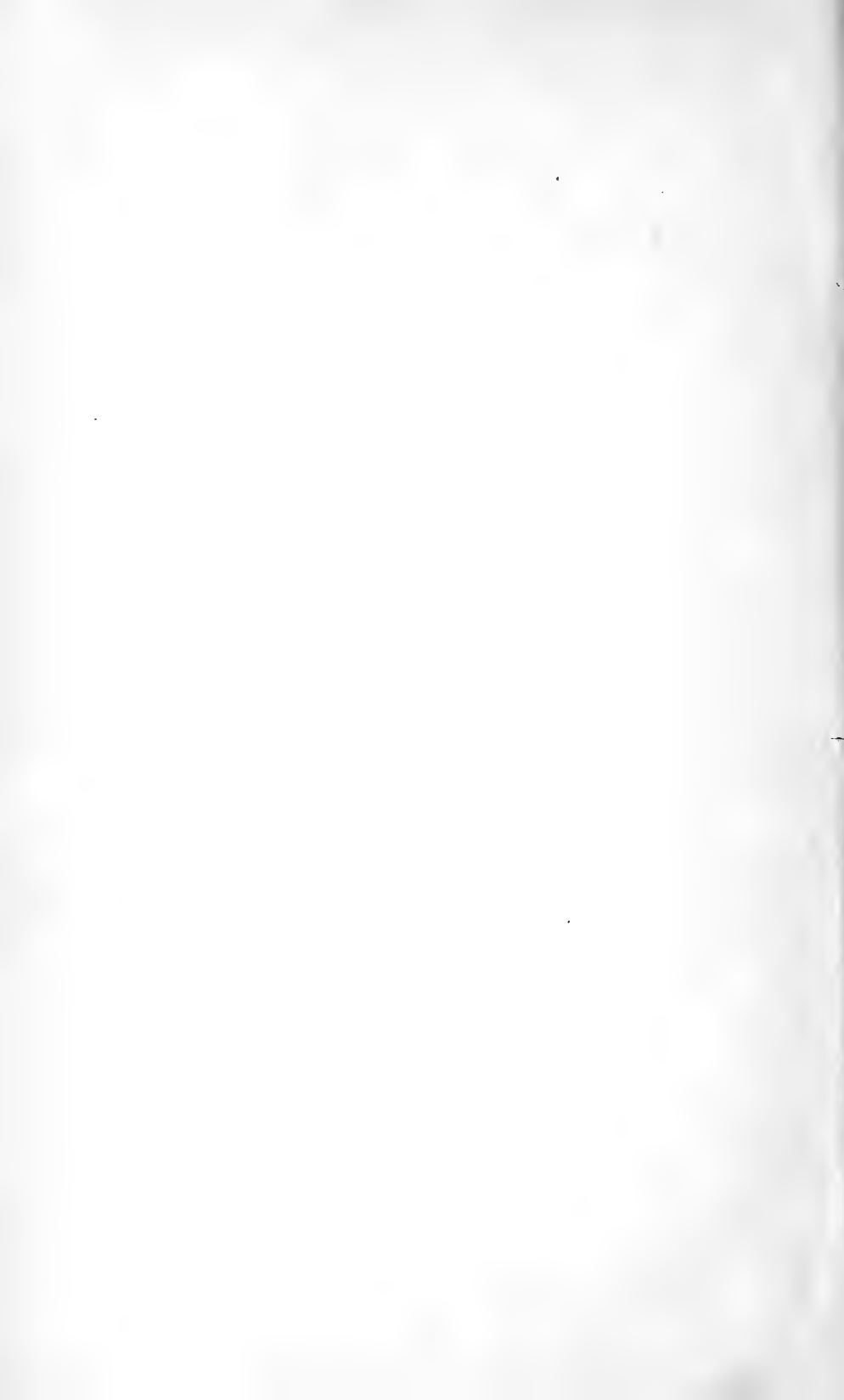


*c*



*d*

Fig. 8.





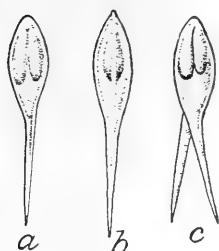


Fig. 1.

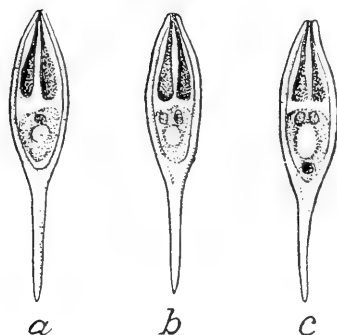


Fig. 2.

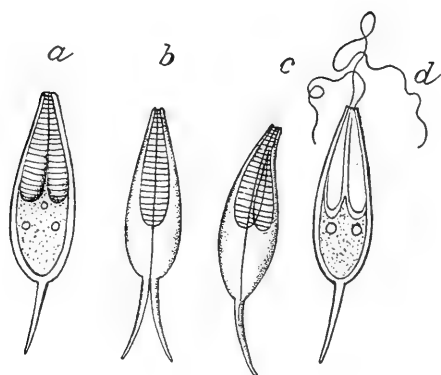


Fig. 4.

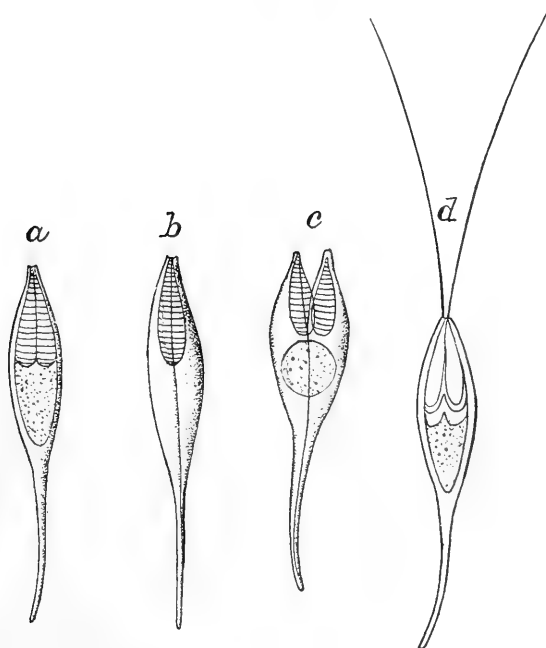


Fig. 3.



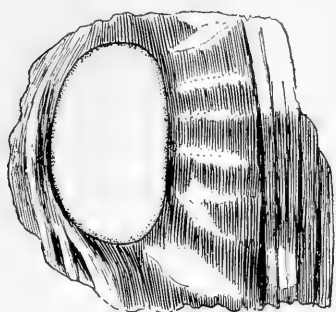


Fig. 1.



Fig. 2.



Fig. 3.

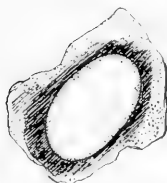


Fig. 4.



Fig. 5.



Fig. 6.

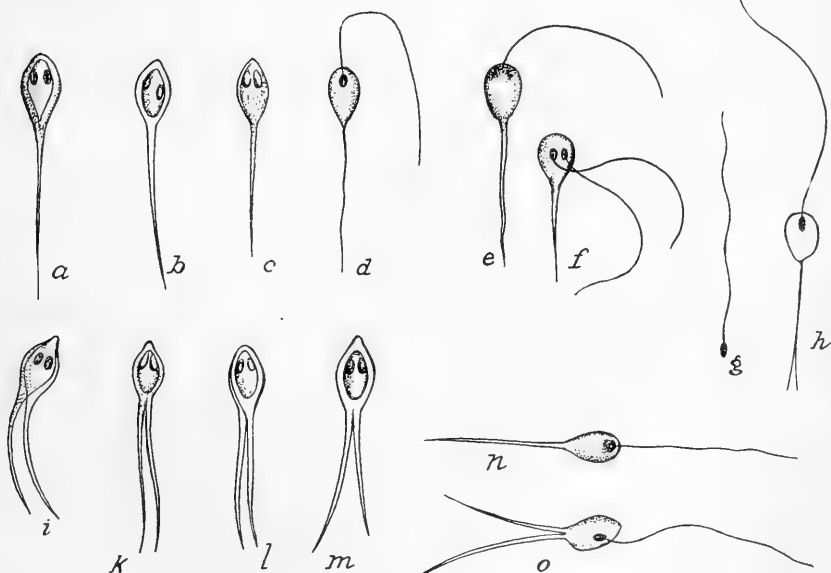
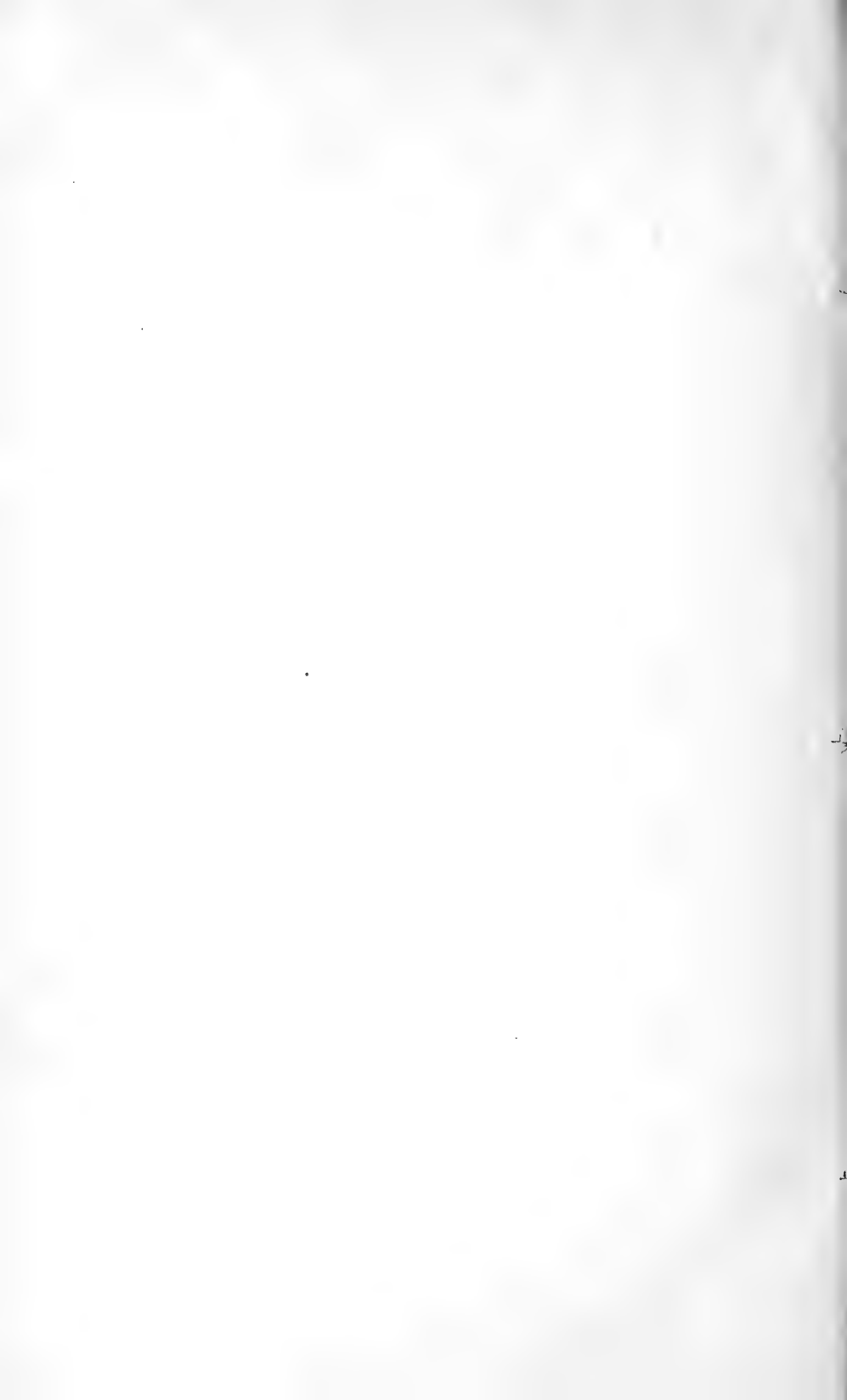


Fig. 7.



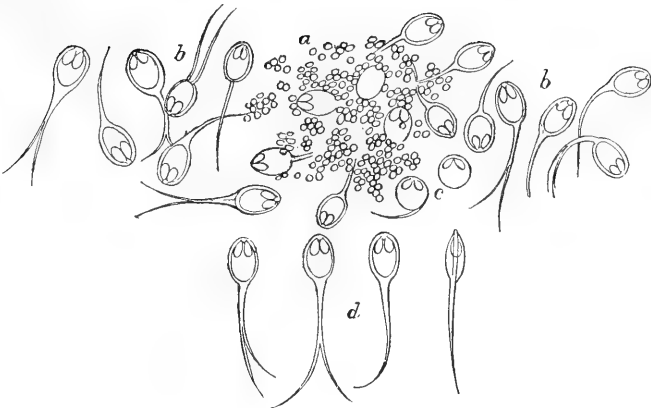


Fig. 1.

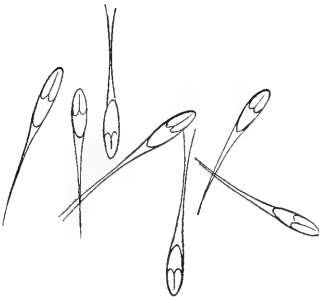


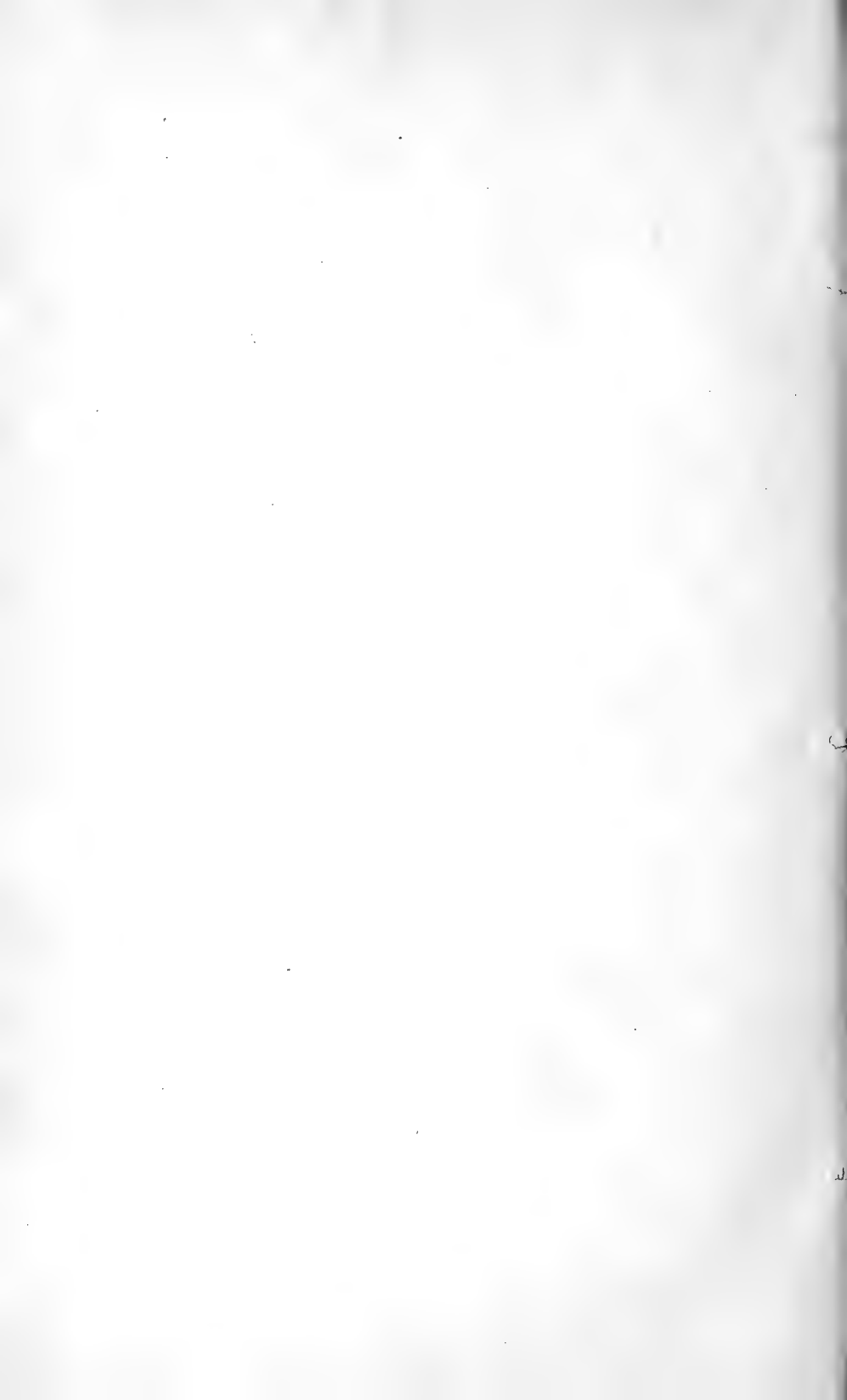
Fig. 2.



Fig. 3.



Fig. 4.



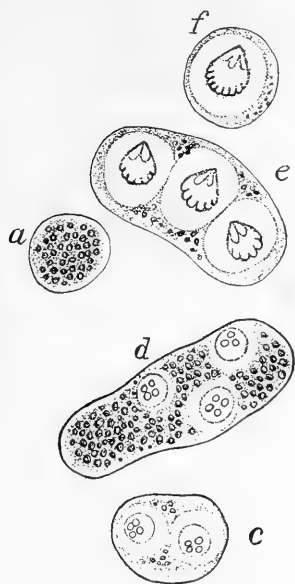


Fig. 1.

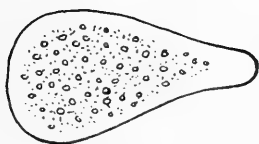


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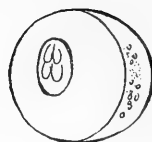


Fig. 5.

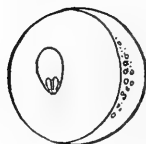


Fig. 6.



Fig. 7.

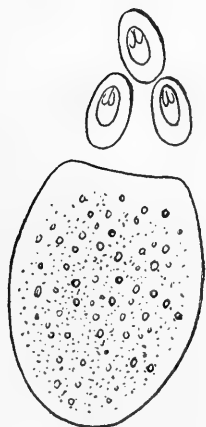


Fig. 4.

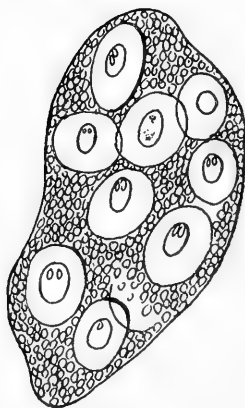


Fig. 3.





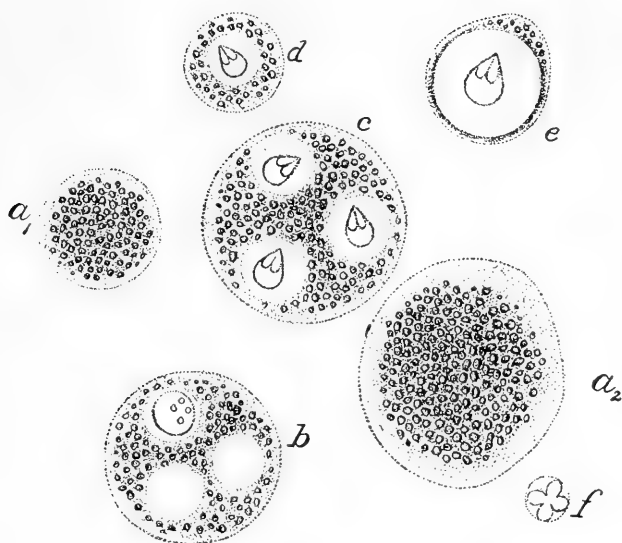


Fig. 1.

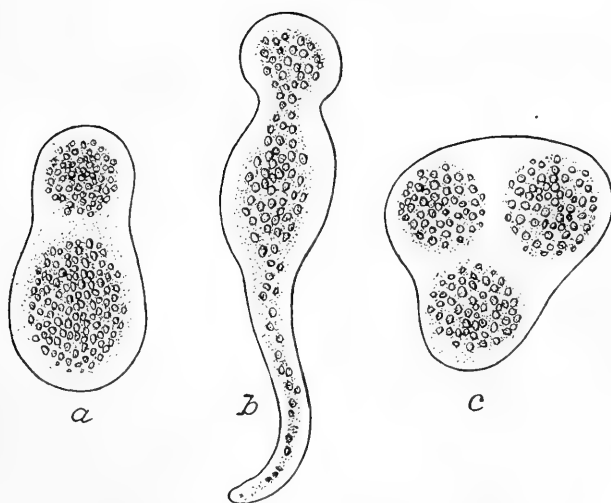


Fig. 2.



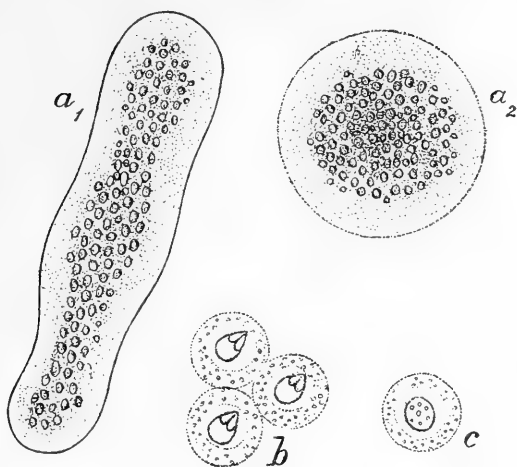


Fig. 1.

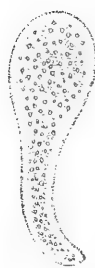


Fig. 3.

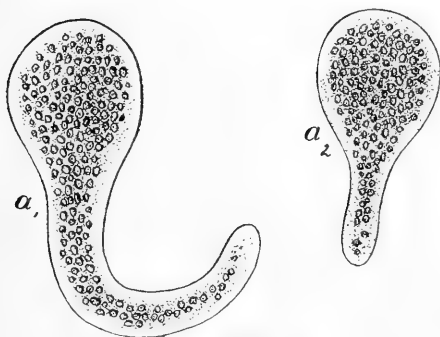


Fig. 2.



Fig. 4.



a

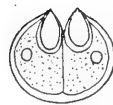


b

Fig. 5.



a



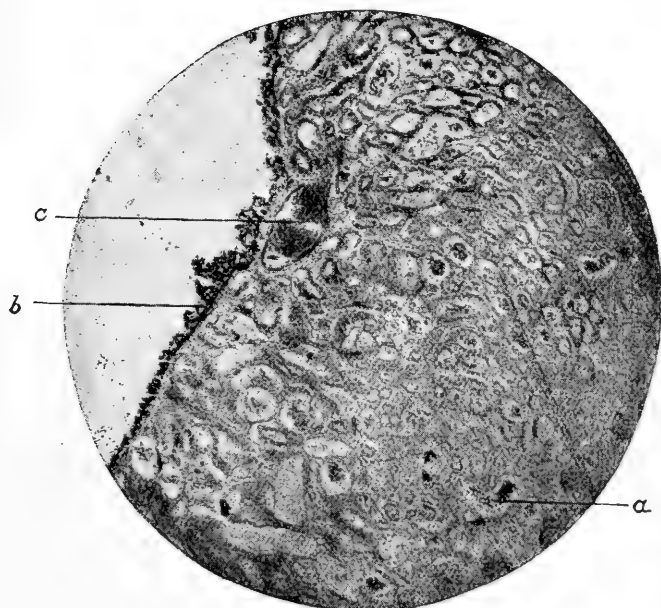
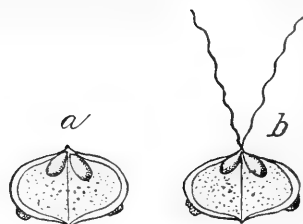
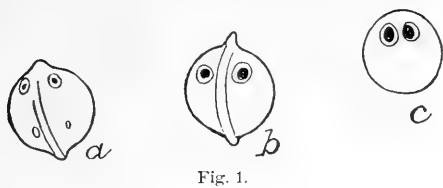
b



c

Fig. 6.







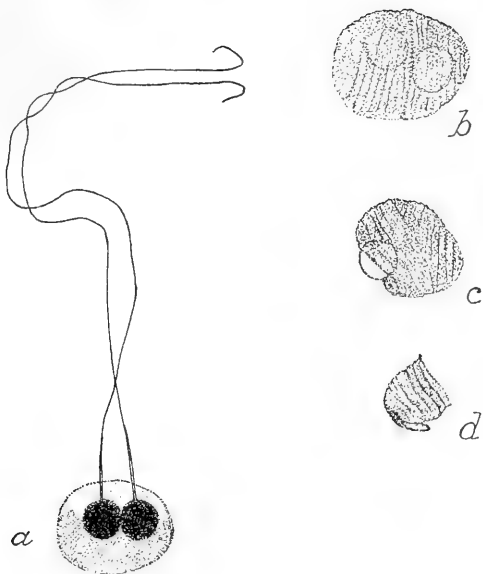


Fig. 1.

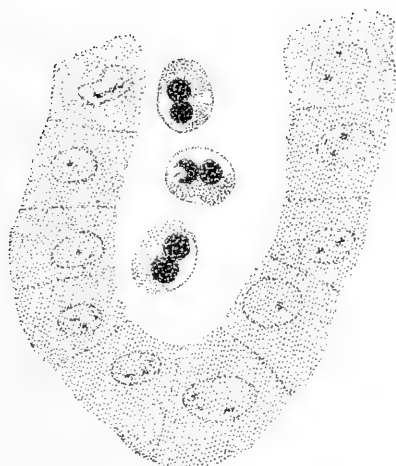


Fig. 2.

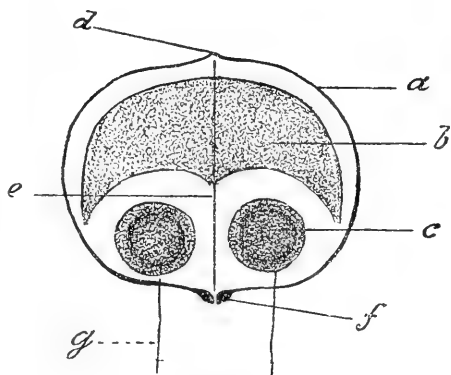


Fig. 3.

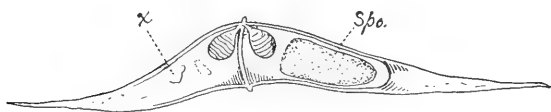


Fig. 4.







Fig. 1.

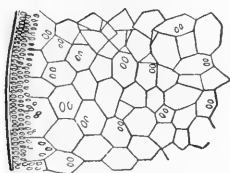


Fig. 2.

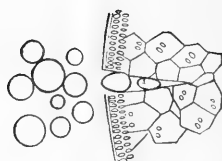


Fig. 3.



Fig. 7.



Fig. 4.



Fig. 5.



Fig. 6.



Fig. 8.



Fig. 9.



Fig. 10.



Fig. 13.



Fig. 11.

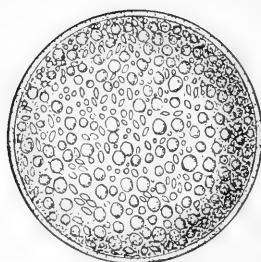


Fig. 12.





Fig. 1.



Fig. 2.



Fig. 4.

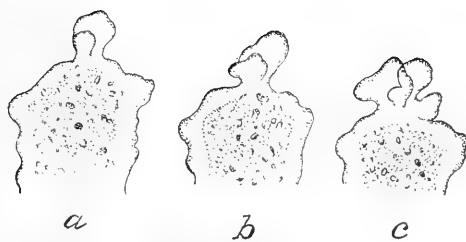


Fig. 3.



Fig. 5.



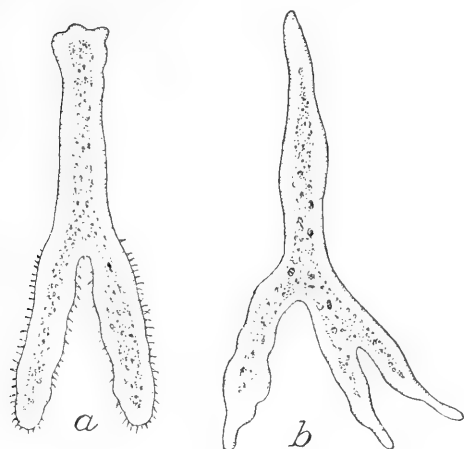


Fig. 1.



Fig. 2.

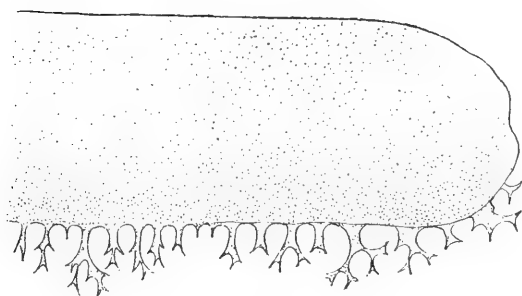


Fig. 4.

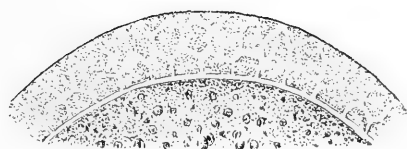


Fig. 3.

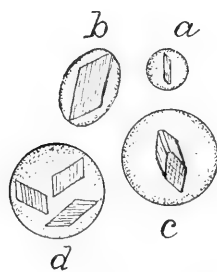


Fig. 5.



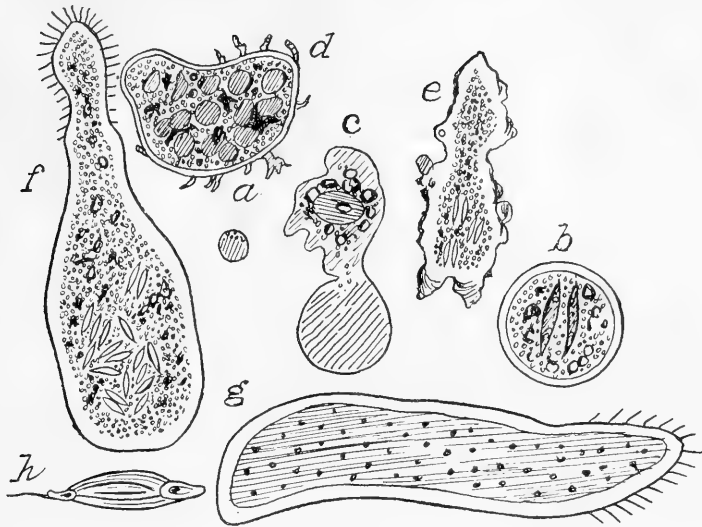


Fig. 1.

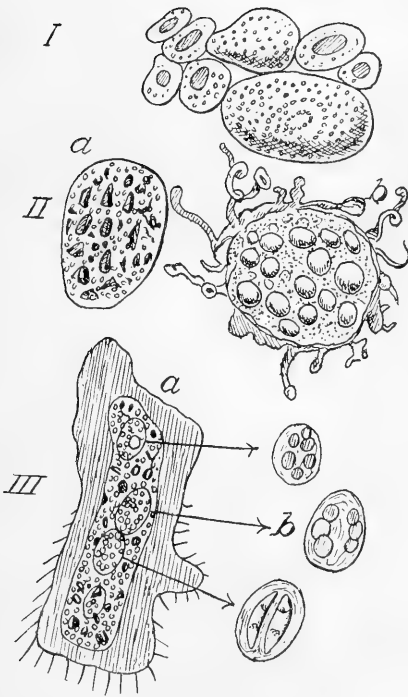


Fig. 2.

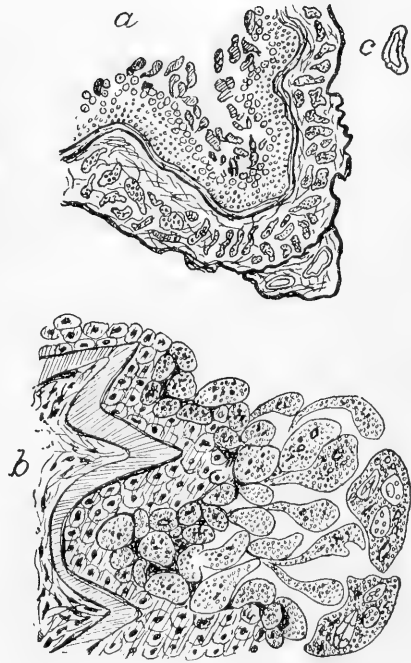


Fig. 3.





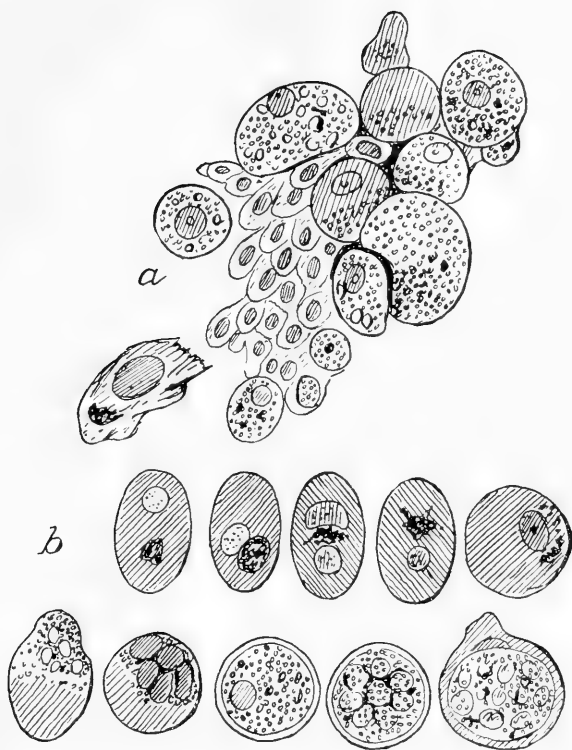


Fig. 1.

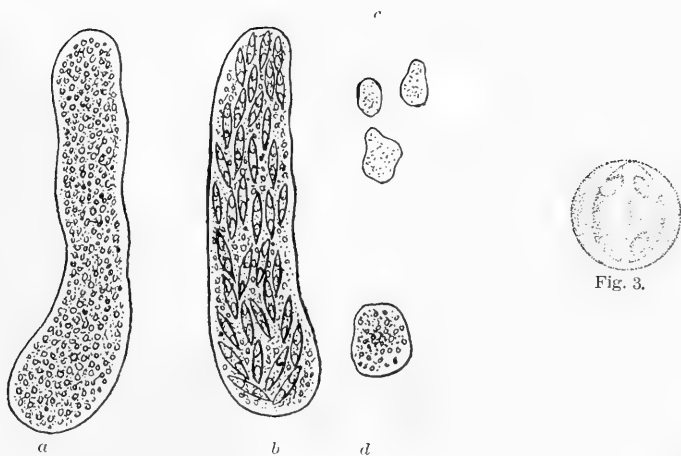


Fig. 2.

Fig. 3.



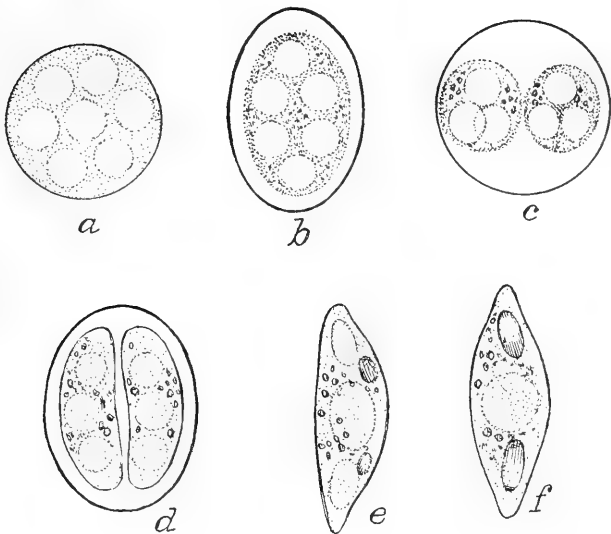


Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



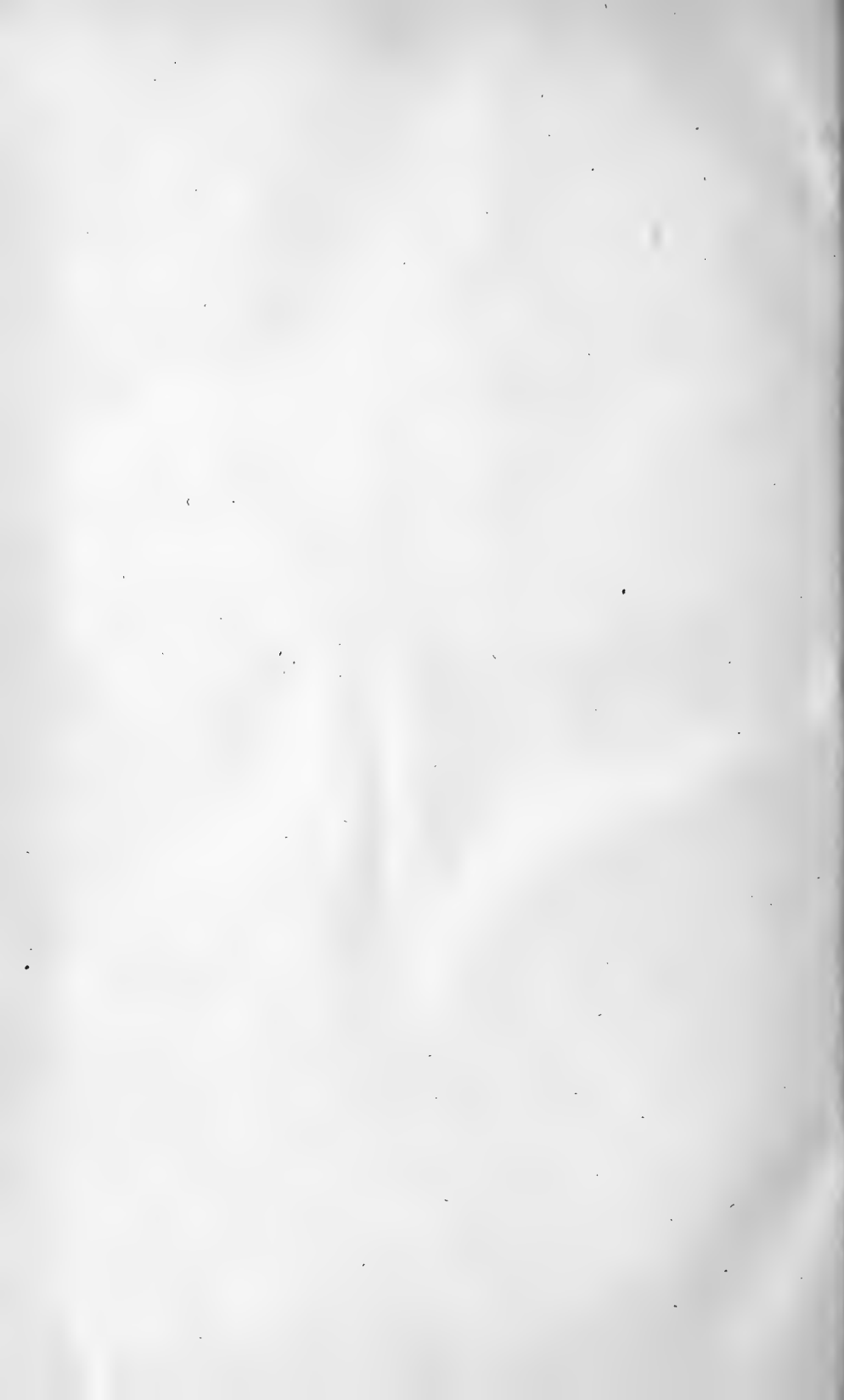
Fig. 5.



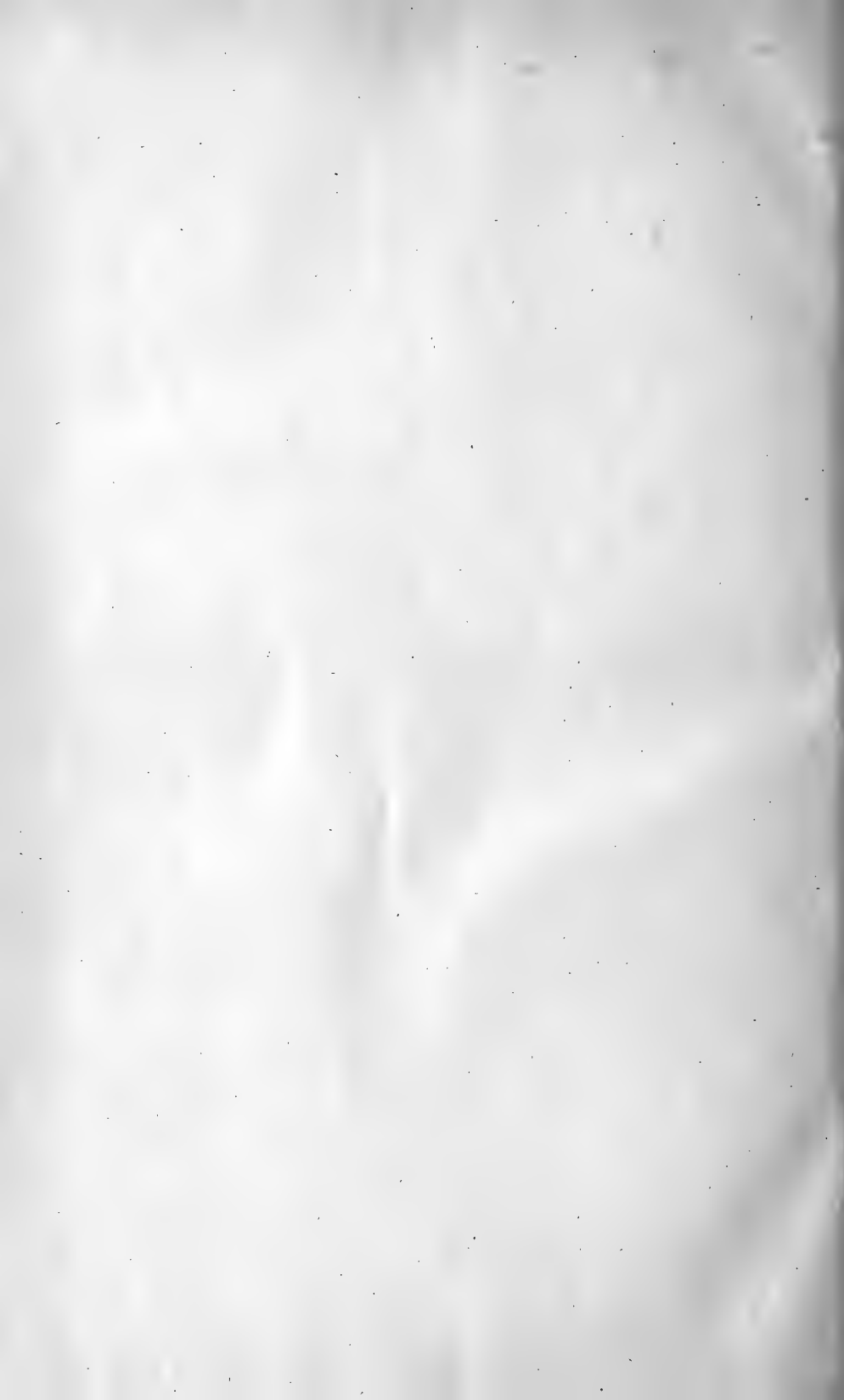
Fig. 6.



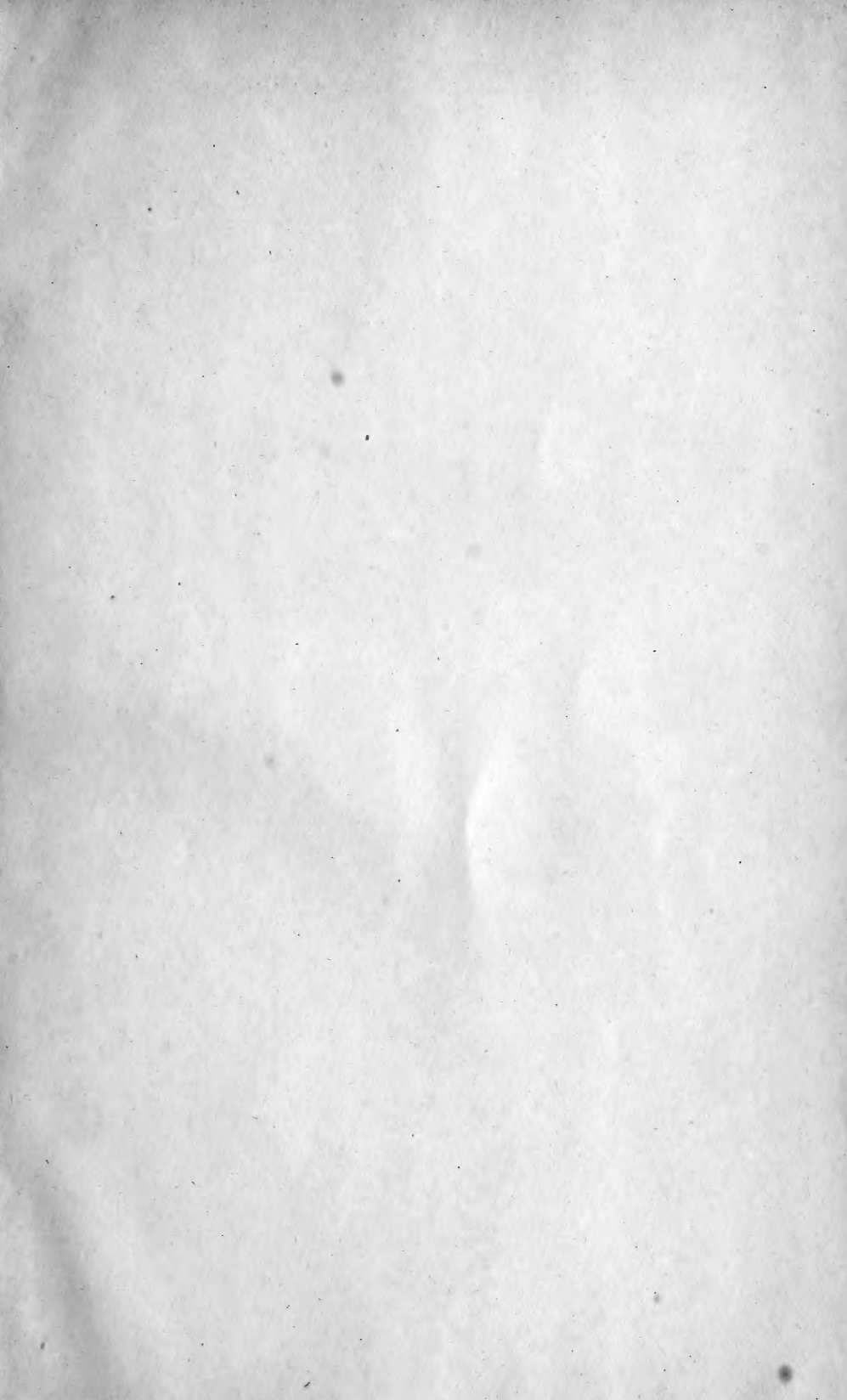


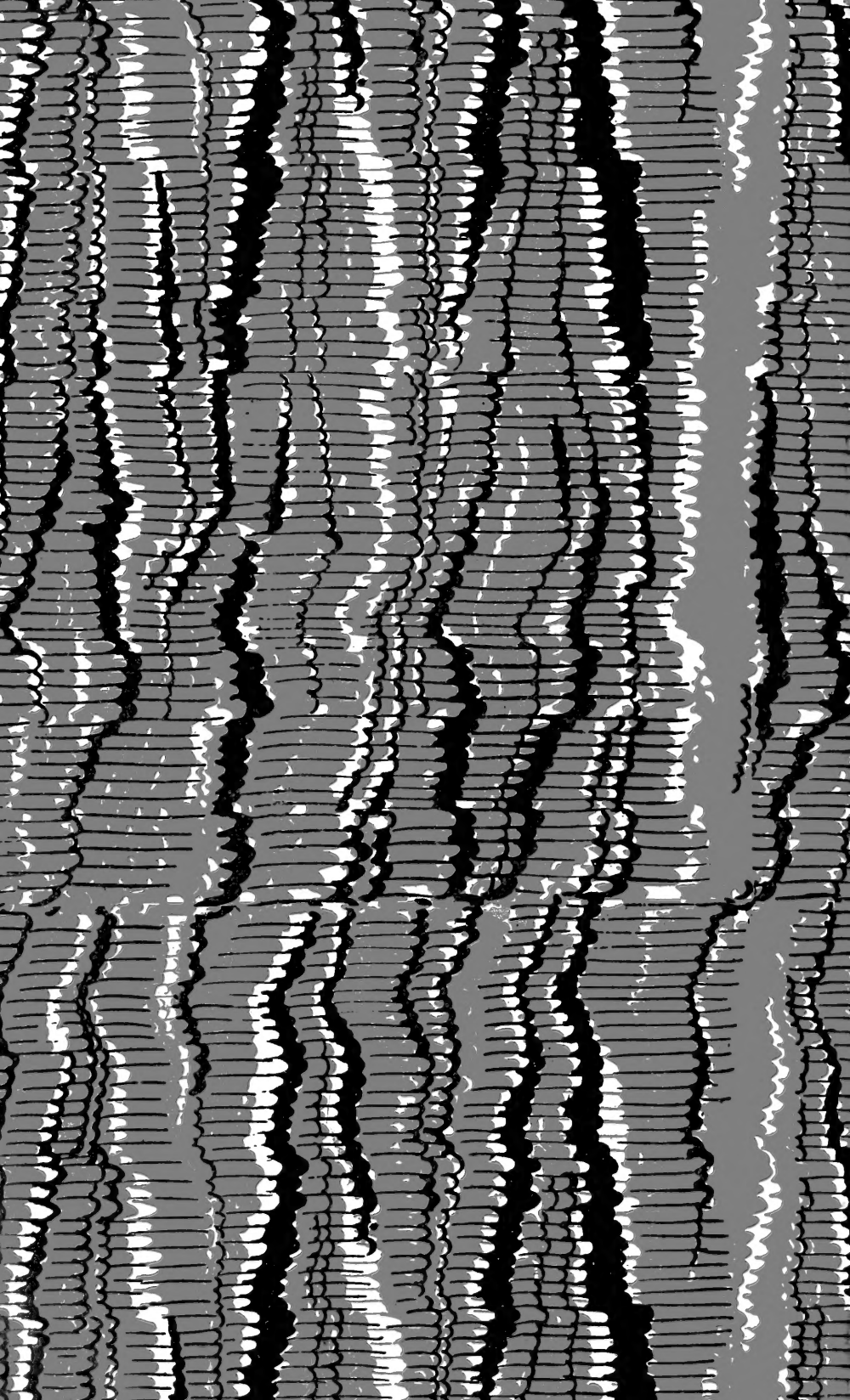


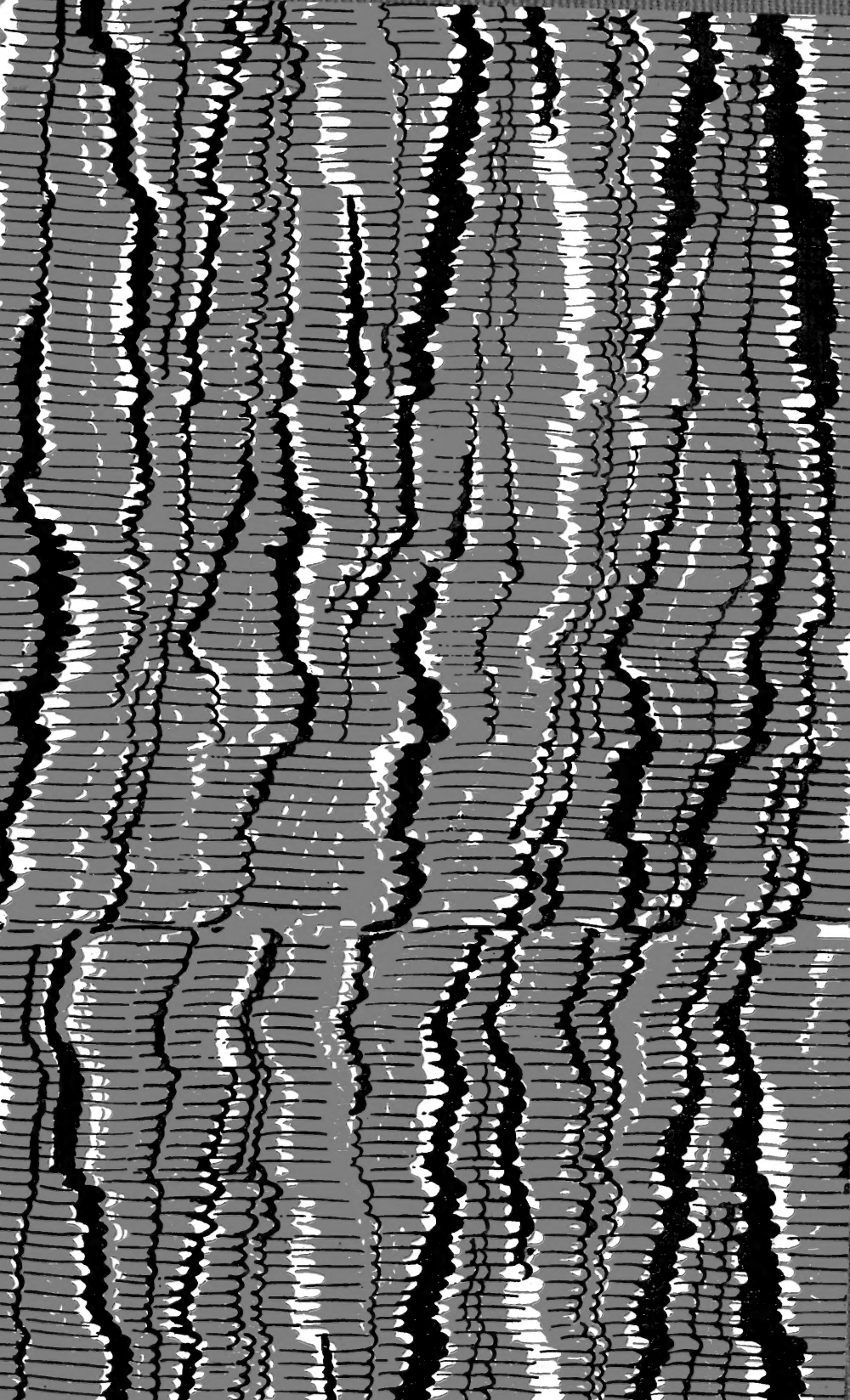












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